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VOLUME 37 JUNE 1959 NUMBER 3

A MICROSPORIDIAN PARASITE OF THE FOREST TENT CATERPILLAR, MALACOSOMA DISSTRIA HBN.[1]

H. M. THOMSON

Abstract

The life cycle of a microsporidian parasite of the forest tent caterpillar is described. The microsporidian attacks primarily the silk glands and the mid-gut epithelium but other tissues are also infected. Observations are made on the ejection of the polar filament. It is apparent that the microsporidian is a new species and the name *Perezia disstriae* n. sp. is proposed.

Introduction

The insect disease survey conducted by the Forest Biology Division of the Canada Department of Agriculture revealed the presence of a microsporidian parasite in the forest tent caterpillar in 1952. The taxonomy and pathogenicity of this parasite remained unstudied until a method of rearing the host insect was developed at the Laboratory of Insect Pathology in 1957. Several egg masses from populations in Ontario were found to be heavily infected by the microsporidian. That these infections arose from contamination of the egg masses by infected females appears to be most likely as no other sources of infection were apparent in the laboratory.

Methods

Stages in the life cycle of the parasite were most easily examined in Giemsa-stained smears. Sections of infected insects were fixed in alcoholic Bouin's solution and stained with Heidenhain's haematoxylin. The living parasite was examined in crushed preparations by dark-field and phase microscope.

Symptoms

Externally, infected larvae exhibited no signs of infection; even after death the larvae were not discolored or deformed. Internally, the infected larvae could be distinguished by the creamy-white color of many areas of the silk glands in contrast with the opalescent appearance of the silk glands of healthy larvae. Microscopically, the creamy-white areas were found to be masses of

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Contribution No. 519 from the Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.

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microsporidian spores. The entire cytoplasm of the cells was replaced by spores and the cells were slightly hypertrophied. Tissues other than the silk glands were also infected but did not display the gross symptoms of the silk glands. The mid-gut epithelium was heavily infected and minor infections were observed in the basement membrane, Malpighian tubules, epidermis, fat body, and tracheae. The silk secretion of infected larvae also contained many spores.

Life Cycle

As the Microsporidia are obligate intracellular parasites, following the course of their development by direct observation is difficult. Stained sections and smears must be used and the stages so observed arranged in sequence. The correct sequence is obscured by the fact that shortly after infection almost

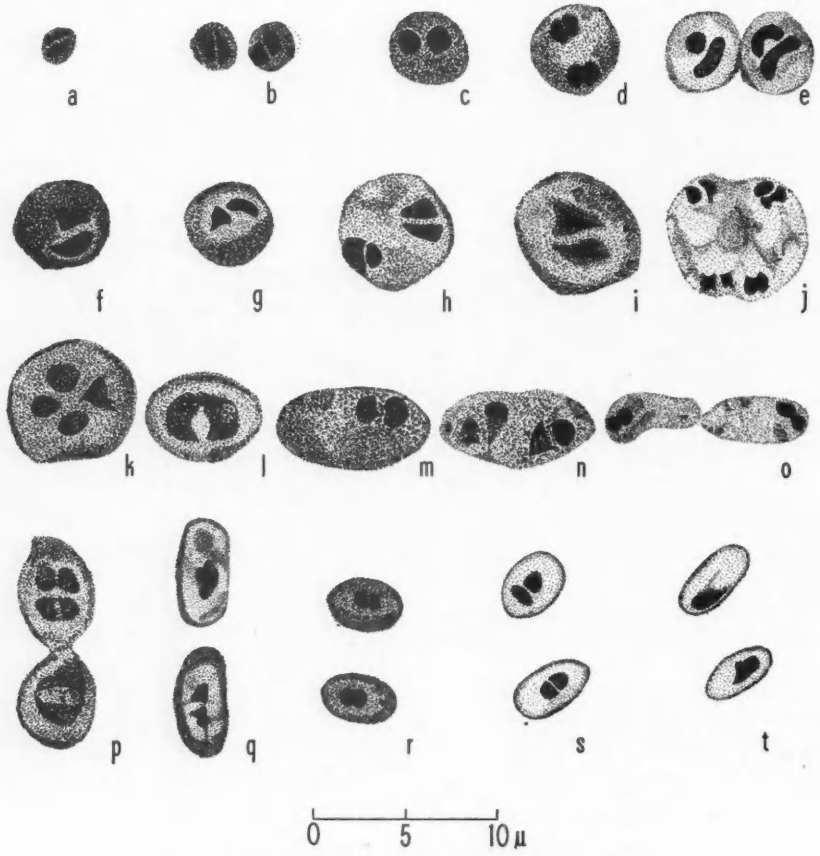

FIG. 1. Presumed life cycle of *Perezia disstriae* n. sp.

all the developmental stages may be found simultaneously. Therefore, the life cycle suggested here is derived by arranging the observed stages in a logical order based on the present knowledge of cell division and nuclear change. By starting with the spore stage, which is known to be the end point of the cycle, and tracing the cycle step by step, the following sequence is suggested.

The earliest schizonts observed measure approximately 1.5 μ. They contain two compact nuclei that all but fill the cell (Fig. 1*a*). The small schizonts grow and divide many times increasing in size and number (Figs. 1*b*–1*k*). The most common method of division is simple binary fission, the nuclei dividing first to form a tetranucleate form (Fig. 1*d*) which then divides into two binucleate forms (Fig. 1*e*). Uninucleate forms were not observed. Occasionally seen were multinucleate schizonts (Fig. 1*j*) containing up to eight nuclei, which are believed to fragment into binucleate schizonts. Chain forms, as observed in many other species of Microsporidia, were not seen.

As the schizonts increase in size, the cytoplasm stains less deeply and becomes more vacuolated and the nuclei develop clear centers. The fully developed schizont measures approximately 7μ in diameter. It is uncertain whether the binucleate (Fig. 1*i*) or tetranucleate (Fig. 1*k*) form is the ultimate schizont.

The sporogonic stages are all more or less elongate; the cytoplasm is conspicuously vacuolated and the nuclei are not as compact as those of the schizonts. The early sporont is a binucleate oval from (Fig. 1*l*). This stage appears to elongate (Fig. 1*m*) and the nuclei divide (Fig. 1*n*), two going to each end of the cell which constricts at the mid-line (Fig. 1*o*). In some cases the nuclei seem to divide again before cell fission occurs, resulting in a form as shown in Fig. 1*p*. The sporont divides to form two sporoblasts either binucleate or tetranucleate (Fig. 1*q*). The tetranucleate condition appears to be only temporary, for as the sporoblast matures, the binucleate condition reappears (Fig. 1*r*), the cytoplasm ceases to stain, and a spore wall appears (Fig. 1*s*). Apparently, the nuclei fuse, as the mature spore contains only a single indistinctly shaped nucleus (Fig. 1*t*). Stained spores measure 1–2$\times$3–4 μ.

Spore

Living spores measure 2$\times$4–5 μ. Spores suspended in water are highly refractive and little can be seen aside from a vacuole of variable size at one end of the spore. However, if the spores are first dried and then flooded with water, many lose their refractivity and some internal structure may be observed if viewed by phase microscope. At first a single dark body may be observed near one end of the spore. If observations are continued on water suspensions of spores little else is observed; however, if liver extract, host gut fluid, or haemolymph is used in place of water the dark body may disappear and what appears to be two nuclei become visible. This process may take several hours and varies considerably between spores; in some cases the two nuclei are apparent as soon as the refractivity disappears and in others the single dark

body remains unchanged. The meaning of these changes is not clear but they may be part of a maturation or germination process. Similar observations have been made on the spore of the budworm microsporidian *Perezia fumiferanae* Thom.

Observations on the Ejection of the Polar Filament

Ejection of the polar filament from the spores of the forest tent caterpillar microsporidian may be accomplished in several ways. Pressure on the cover slip will cause discharge of the polar filaments but these remain tightly coiled in the vicinity of the spore, making measurements difficult. Application of 3% hydrogen peroxide to the spores will cause filament discharge; however, the liberation of bubbles of oxygen under the cover slip causes considerable turbulence, making observation difficult. Perhaps the most effective way of observing the filament and its discharge is to dry the spores on a slide for several hours and then flood them with a solution of commercial liver extract or a solution of vitamin B-12. It was found that the active principle was not either the liver extract or the vitamin B-12 but rather the 0.5% phenol contained in these solutions as a preservative. As a result of this discovery a large number of phenolic derivatives were tested for ability to evoke filament discharge. Of these, benzaldehyde, pyrocatechol, benzyl alcohol, phloroglucinol, and phenylenediamine showed signs of activity. None, however, was as effective as phenol. Although the phenolic solutions cause filament discharge from only a small percentage of spores, they are none the less useful as it is possible to examine thousands of spores within a short time. The filaments are difficult to see by means of ordinary light microscopy, and a phase microscope is invaluable.

Observations on filament discharge under the influence of liver extract are as follows: approximately 20 minutes after the slide is flooded a slight protuberance may be observed at one end of the spore; this is the site of filament discharge. Forty minutes after the slide is flooded the spores start to discharge their filaments; the process occurs very quickly and is accompanied by vigorous vibration of the spore. The filament shoots out at great speed but may stop momentarily and then proceed in a different direction. Frequently the filament is at first spiral but usually straightens out within a few moments. The observation does not support the observations of Dissanaike (1) and Krieg (2) that the filament is "whipped out" with the distal end emerging from the spore last. One receives the impression that the filament is growing from the spore rather than being extruded.

The filaments vary in length from 70 to 120 μ, the average being 95 μ. Immediately upon filament discharge, a globular structure can be seen at the tip of the filament. Such structures can also be seen in spore preparations treated with hydrogen peroxide but are difficult to detect as they disintegrate almost as soon as they appear. The phenolic compounds are also poor media for studying these structures; liver extract is much superior, the globules

persisting for 3 minutes or more before swelling and disappearing. Good stained preparations have not been obtained, the structures being apparently too delicate for the process of fixing and staining.

Discussion

As two spores are formed from each sporont and no large cysts are formed within the host tissues, the forest tent caterpillar microsporidian is a member of the genus *Perezia*. A comparison with the other species of *Perezia* described from Lepidoptera (see Table I) reveals sufficient difference to distinguish the forest tent caterpillar microsporidian from any of them. The tissue primarily attacked and the spore size are the most distinctive characteristics. It is apparent that this is a new species and the name *Perezia disstriae* n. sp. is proposed.

TABLE I

A comparison of the more important characteristics of the species of the genus *Perezia* infecting the Lepidoptera

Species	Host	Primary site of infection	Spore size (microns)	Maximum no. nuclei*
P. legeri Paillot (3)	*Pieris brassicae*	Fat body Giant cells	4.0–5.0	8
P. mesnili† Paillot	*Pieris rapae* *Pieris brassicae*	Mid-gut Malpighian tubules	3.4–4.4 × 1.4–2.7	8
P. pieris Paillot (4)	*Pieris brassicae*	Malpighian tubules Silk glands	5.0–6.0	4
P. pyraustae Paillot (5)	*Pyrausta nubilalis*	Malpighian tubules Silk glands	3.5–6.0 × 1.8–3.0	8
P. fumiferanae Thomson	*Choristoneura fumiferana*	Malpighian tubules Mid-gut	3.0–5.2 × 1.7–2.2	12
P. disstriae Thomson	*Malacosoma disstria*	Silk glands Mid-gut	4.0–5.0 × 2.0	8

*In polynuclear schizonts.
†As described by Tanada (6).

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CUTICONEMA VIVIPARA N. G., N. SP., A NEW SAPROPHAGOUS NEMATODE OF THE SUBFAMILY PANAGROLAIMINAE THORNE, 1937, FROM CANADA, WITH A NOTE ON THE GENUS BREVIBUCCA GOODEY, 1935[1]

K. C. SANWAL[2]

Abstract

Cuticonema vivipara n. g., n. sp. (subfamily Panagrolaiminae Thorne, 1937) is described from the roots of an oak tree. The genus is characterized by six flaplike lips separated by grooves with sclerotized borders; panagrolaimoid type of stomatorhabdions; large amphid apertures; an inner cuticle with pores enveloped by an outer cuticle, both striated but without incisures; esophagus divisible into four parts; single prodelphic reflexed ovary, posteriorly situated vulva, presence of uterine glands; single outstretched testis in the male, unequal spicules, rudder-shaped gubernaculum, and numerous caudal papillae in the male tail. The species is ovoviviparous. The new genus is compared with other genera of the Panagrolaiminae and with *Brevibucca* Goodey, 1935.

Introduction

Seventeen male and 20 female nematodes were collected from the roots of a fallen oak tree in the suburbs of Ottawa, Ontario. Almost half of the nematodes were studied while they were living, a portion of the root sample being examined from time to time. Study of the living worms and mounted specimens showed that they represented a new genus of nematodes, which is described herein and is placed in the subfamily Panagrolaiminae Thorne, 1937 (10). The resemblances shown to the type species of the genus *Brevibucca* Goodey, 1935 (4), are discussed. This necessitated a discussion of the taxonomic positions of the two species of *Brevibucca*.

Cuticonema new genus

Diagnosis

Cuticle without lateral incisures; body enveloped by an additional cuticular sheath strongly striated transversely; inner cuticle with longitudinal rows of pores; six flaplike lips separated by deep grooves; stomatorhabdions sclerotized, the posterior ones being surrounded by esophageal tissue; esophagus divisible into precorpus, slightly swollen corpus, isthmus, and valvulated bulb. Amphid apertures large, elliptical; ovary single, prodelphic, and reflexed; vulva posterior; uterine glands present; ovoviviparous. Testis single, outstretched; spicules unequal; gubernaculum rudder-shaped; caudal papillae numerous. Type species: *Cuticonema vivipara.*

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Contribution No. 3896, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Nematode Section, Entomology Laboratory, Ottawa.

Cuticonema vivipara n. g., n. sp.

Female

Length 1.0–1.4 mm; a = 15.8–19.4; b = 4.5–6.5; c = 9.3–15.5; v = 85–88% (number of worms measured, 9).

Worms small and stout. Body gradually attenuated towards both extremities and covered by double cuticle. Outer cuticle thick, strongly striated transversely, and covering whole worm like a sheath, sometimes everted to form funnel-shaped or collarlike structures at the anterior end, or cupped at posterior end of body by retraction of tail (Figs. 8*a*–8*d*). Striations on sheath 0.0017 mm apart (Fig. 6*a*). Inner cuticle thin; smoothly striated transversely; with longitudinal rows of punctations (Fig. 6*b*); with two longitudinal rows of well-defined pores, evenly spaced, along the dorsal and ventral lines of the body (Fig. 5). Lateral incisures absent.

Head not marked off from rest of body; bearing six flaplike, well-defined, and mobile conical lips separated from one another by deep semicircular grooves, the borders of which are sclerotized (Fig. 2). Six small papillae, one at apex and one at base of each lip. In addition, four or probably more setose papillae at bases of lips.

Terminal mouth opening leading into stoma, the walls of which are heavily sclerotized (Fig. 2). The foremost rhabdion has three different regions of sclerotization. Base of this foremost rhabdion rests on a small rhabdion from which it is separate. The small second rhabdion is followed by a third rhabdion, which shows weak sclerotization and is raised into two toothlike structures in front of its junction with the walls of the esophageal lumen. My interpretation of the homologies of the stomatorhabdions is as follows: The three differently sclerotized regions of the anteriormost rhabdion represent the cheilorhabdions, prorhabdions, and mesorhabdions in order, all three fused into a long piece. The second small rhabdion on which the base of this long piece rests represents the metarhabdion. The third, very feebly sclerotized rhabdion, which is raised into two toothlike structures, represents the telorhabdion.

Esophagus broadened considerably at its middle, giving rise to three more or less distinct regions: precorpus, corpus, and isthmus (Fig. 1). Corpus not in shape of a well-defined median bulb, but elongated and somewhat spindle-shaped. Isthmus narrow and terminating in esophageal bulb containing a valve composed of three pectinate elements. Tissue of precorpus enveloping bases of posterior elements of stoma. Tissue of corpus evidently different from that of precorpus and isthmus, from both of which it is demarcated by faint lines. Lumen of esophagus triradiate, the three radii showing clearly to beginning of isthmus.

Well-developed cardia between esophagus and intestine. Lumen of intestine wavy. Walls of intestine composed of large epithelial cells that appear polygonal on surface view. Cell contents granular. Rectum very long and narrow, with thick cuticular lining. Rectal glands present (Fig. 1).

Nerve ring with dorsal tilt, surrounding esophagus at level of beginning of isthmus or slightly posterior (Fig. 1). Amphid apertures large and elliptical, situated posterior to bases of lips (Fig. 2). Phasmids slightly asymmetrical in position, about 0.09 mm from tip of tail (Figs. 1, 7*a*, 7*b*).

Excretory pore almost at level of nerve ring, leading into narrow duct directed posteriorly (Fig. 1). Two glands with finely granular contents and each with a large nucleus, situated ventrad of esophageal bulb. Link between these and excretory duct not observed.

Ovary single, small, and reflexed (Fig. 1). Oöcytes in single row. Generally one large unfertilized egg at the proximal end of the ovary; oviduct short and narrow. First part of uterus functioning as seminal receptacle. Uterus proper large and broad, extending as a wide tube towards posterior end, where it narrows slightly. Vagina long, narrow, and slightly curved; walls lined with thick cuticle. A pair of uterine glands, with granular cytoplasm and a large nucleus in each, situated at level of terminal part of uterus and probably opening into vagina. Vulva a transverse, slitlike aperture without elevated lips. Radiating muscle fibers observed with ends attached to body wall and vulvar lips. In vulvar region, outer cuticular sheath forming a peculiar pouchlike structure enveloping vulva within its fold (Figs. 1, 7*a*, 7*b*), and in a living worm, giving appearance of a caudal sucker. Eggs large, observed in different stages of development, from two-celled stage to a fully formed larva. Ovoviviparous. Eggs hatching within uterus, and a large number of larvae (3–11) observed in terminal part of uterus. Total number of eggs in uterus varying from 8 to 13 at one time.

Outer cuticular sheath forming an elevated, round, suckerlike structure encircling the anal aperture (Fig. 7*a*). Tail short and gradually attenuated to a conical point.

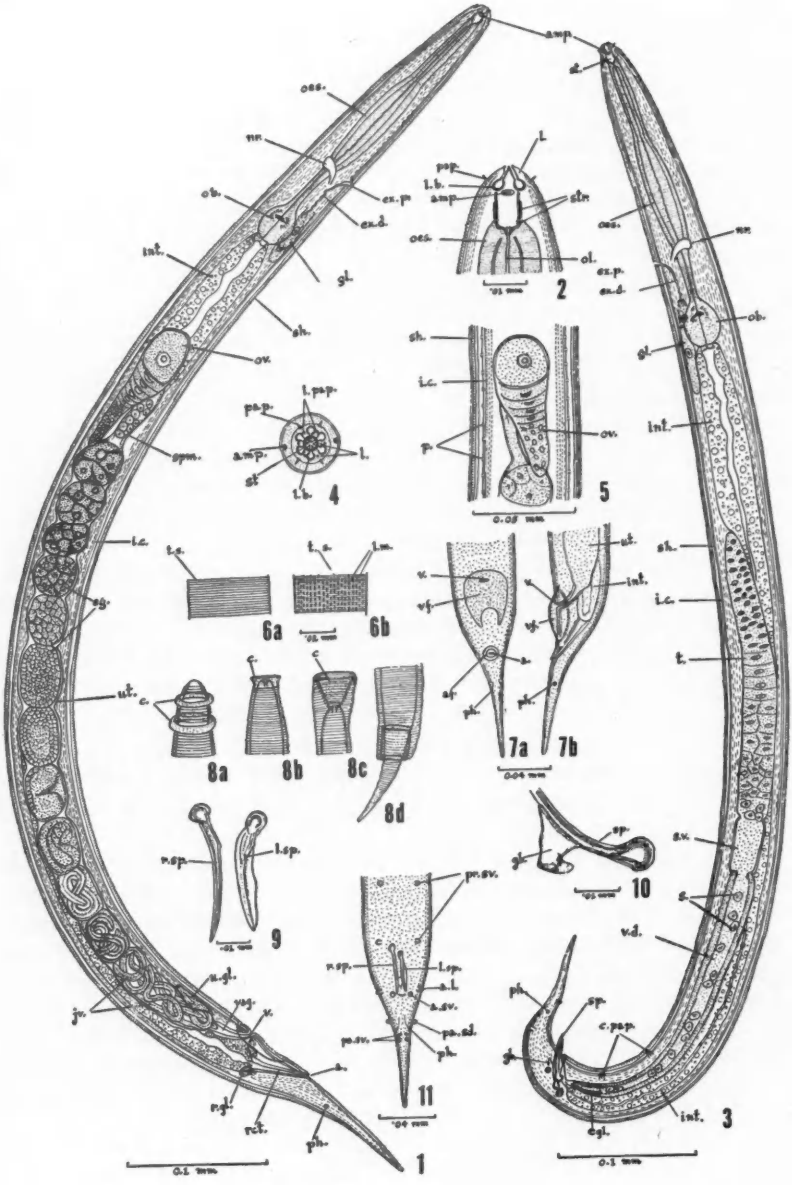
Male

Length 0.9–1.4 mm; a = 18.8–21.1; b = 3.9–5.2; c = 9.3–12.7; left spicule 0.033–0.037 mm; right spicule 0.044–0.046 mm; gubernaculum 0.013 mm (number of worms measured, 9).

Cuticle, cuticular pores, head, lips, papillae, stoma, esophagus and its subdivisions, nerve ring, and excretory pore similar to those in female.

Striations on outer cuticle 0.00178 mm apart, and lateral incisures absent. Intestine narrowed along the testis and vas deferens. Rectal glands not clearly observed.

Single testis extending anteriorly beyond middle of body and not reflexed (Fig. 3). Spermatocytes in single row first, in double rows later. Vas deferens differentiated into two regions; posterior end narrowed considerably with two or three elongate, finger-shaped, glandlike structures with granular contents associated with it; glandlike structures evidently closely apposed to the walls of vas deferens. Vas deferens observed filled with sperms. Two unequal spicules, the right one longer and slenderer than the left, which has a strongly sclerotized lateral expansion (Figs. 9, 11). Both spicules with prominent heads. Gubernaculum rudder-shaped (Fig. 10).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Tail region curved. Tail short and gradually attenuated to fine point. Seven pairs of caudal papillae as follows: Two pairs preanal, both subventral; two pairs adanal, one being subventral and the other lateral (both or either of these adanal pairs may be slightly pre- or post-anal in some specimens); three pairs postanal, one being subdorsal and two subventral. Phasmids midway between subdorsal pair and penultimate postanal pair (Fig. 11).

Discussion

The newly described form shows affinities to two subfamilies, Panagrolaiminae Thorne, 1937 (10), and Rhabditinae Chitwood and Chitwood, 1937 (1). Its anatomical peculiarities suggest that it bridges the gap between the familes Cephalobidae and Rhabditidae. The affinities towards the genera of the Panagrolaiminae, however, are greater except for one genus of the Rhabditinae, to be discussed shortly, and the genus is placed in the Panagrolaiminae provisionally until more species may be discovered and the taxonomic position can be more exactly evaluated.

The characters that favor its inclusion in the Panagrolaiminae are: stomatorhabdions similar in general pattern to those of various genera of Panagrolaiminae (i.e., without attempting to interpret and homologize the different parts in terms of the conflicting terminologies of different workers); pharyngeal musculature enveloping posterior elements of stoma; a single ovary, reflexed straight back without additional flexures; uterus functioning as seminal receptacle; and rudder-shaped gubernaculum. The lips bear some resemblance to those of the genus *Panagrolaimus* Thorne, 1939, in that both genera have lips with strongly sclerotized borders that are deeply notched; however, they differ in shape and size in the two genera. The genus resembles *Procephalobus* Steiner, 1934, in that both genera possess an esophagus with a slightly swollen corpus. Further, the general pattern of the distribution of the caudal papillae in the male tail of the present form resembles closely that of the genera *Plectonchus* Fuchs, 1930, *Tricephalobus* Steiner, 1936, and *Panagrobelus* Thorne, 1939. The resemblances to the above genera are restricted, however,

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FIGS. 1–11. *Cuticonema vivipara* n. g., n. sp. 1. Female, general anatomy. 2. Anterior region of female. 3. Male, general anatomy. 4. Head of female, *en face* view. 5. Ovarian region of female, pores through inner cuticle. 6*a*. Transverse striations of outer sheath. 6*b*. Transverse striations and longitudinal punctations of inner cuticle. 7*a*, 7*b*. Ventral and lateral views respectively of posterior region of female, cuticular formations in region of vulva and anus. 8*a*–8*d*. Collarlike and variously shaped structures formed by cuticular sheath at anterior and posterior ends of body. 9. Spicules. 10. Gubernaculum. 11. Ventral view of male tail.

ABBREVIATIONS: *a.*, anus; *a.l.*, adanal lateral papilla; *a.sv.*, adanal subventral papilla; *af.*, cuticular formation around anus; *amp.*, amphid; *c.*, cuticular collar; *c.pap.*, caudal papillae; *eg.*, egg; *egl.*, ejaculatory gland; *ex.d.*, excretory duct; *ex.p.*, excretory pore; *gb.*, gubernaculum; *gl.*, glands (probably associated with excretory system); *i.c.*, inner cuticle; *int.*, intestine; *jv.*, larvae; *l.*, lip; *l.b.*, lip border; *l.m.*, longitudinal markings; *l.pap.*, labial papillae; *l.sp.*, left spicule; *nr.*, nerve ring; *ob.*, esophageal bulb; *oes.*, esophagus; *ol.*, esophageal lumen; *ov.*, ovary; *p.*, pores; *pap.*, setose papillae; *pa.sd.*, postanal subdorsal papilla; *pa.sv.*, postanal subventral papillae; *ph.*, phasmid; *pr.sv.*, preanal subventral papillae; *r.gl.*, rectal glands; *r.sp.*, right spicule; *rct.*, rectum; *s.*, sperms; *s.v.*, seminal vesicle; *sh.*, cuticular sheath; *sp.*, spicule; *spm.*, spermatheca; *st.*, stoma; *str.*, stomatorhabdions; *t.*, testis; *t.s.*, transverse striations; *ut.*, uterus; *u.gl.*, uterine gland; *v.*, vulva; *v.d.*, vas deferens; *vag.*, vagina; *vf.*, cuticular fold around vulva.

to the characters mentioned. With these exceptions, the present form differs from all other genera of the Panagrolaiminae in the following characters: adult worm with a living, transversely striated cuticular sheath which is mobile, eversible at anterior end of body, and capable of forming collarlike structures of varying shapes; lateral lines absent; cuticular pores present along the dorsal and ventral body lines; large elliptical amphidial openings situated back of lips in region of stoma; shape and structure of lips; typical arrangement of the stomatorhabdions as described above; esophagus with a long median swelling; vulva posterior in position; uterine glands present; unequal spicules and ovoviviparous habit of reproduction.

On the basis of the foregoing differences the new form is designated as a new genus, with *Cuticonema vivipara* as the type species.

C. vivipara resembles closely the type species of the genus *Brevibucca* Goodey, 1935 (4), which has been placed in the subfamily Rhabditinae. Dougherty (1955)(2) accepted it only provisionally in the Rhabditinae, placing it as 'incertae sedis'. In the addendum to the same paper, however, he remarked that *Brevibucca* and *Poikilolaimus* Fuchs, 1930 (3), are forms that tend to bridge the gap between the Rhabditidae and the Cephalobidae and he suggested the new subfamily Poikilolaiminae to embrace these two genera, placing this new family in the Rhabditidae. *Poikilolaimus*, it should be noted, has been placed in the Rhabditinae by Ruehm (1956)(8), and in the Panagrolaiminae by Skrjabin *et al.*, 1954 (9), as a synonym of *Panagrolaimus* Fuchs, 1930, though Goodey (1951)(6) and Chitwood (1950) made no mention of it. Paramonov (1956)(7) has created a new subfamily, Brevibuccinae, for the genus *Brevibucca*.

The marked differences between the two species of *Brevibucca* seem to indicate that the two species may belong to two different genera. These differences are as follows: The irregularly shaped rhabdion (interpreted as telorhabdion by Goodey), and the small prominences on the base of stoma (interpreted as part of esophagus by Goodey but considered part of stoma by the writer) in *Brevibucca saprophaga* Goodey, 1935 (4), are absent in *B. frugicola* Goodey, 1942 (5). *B. saprophaga* has one ovary, a posteriorly situated vulva, a precorpus merging with the corpus, unequal spicules, and uterine glands. In contrast with the above, *B. frugicola* has two ovaries, an equatorial vulva, a well-defined esophageal bulb, equal spicules, and no uterine glands. *B. frugicola* has distinct lateral fields and well-developed caudal alae, which are absent in *B. saprophaga*. On the basis of the above differences, it appears to be more tenable to split the existing genus *Brevibucca* into two genera: *Brevibucca* Goodey, 1935 (4), with *B. saprophaga* as type species and generic diagnosis as given by Goodey, 1935 (4), and another genus (incertae sedis) with the existing *B. frugicola* as the type species. This new genus could well be retained in the Rhabditinae and the genus *Brevibucca* could either be moved over to the Cephalobidae (probably under the Panagrolaiminae), or accommodated in the subfamily Brevibuccinae Paramonov, 1956, after emending the characters of the subfamily.

Cuticonema vivipara resembles *Brevibucca saprophaga* in absence of lateral lines, possession of single reflexed ovary, uterine glands, unequal spicules, and posterior position of vulva. *C. vivipara*, however, differs from *B. saprophaga* in having six flaplike lips, deeply notched and with sclerotized borders in contrast with six low-lying lips in *B. saprophaga*. The amphid apertures in *C. vivipara* are large, elliptical, and situated more posteriorly than the small, slitlike amphid apertures in *B. saprophaga*, which are situated at the base of the lips. The excretory pore in *C. vivipara* lies at the level of the nerve ring in contrast with the excretory pore lying in front of the nerve ring in *B. saprophaga*. There is a permanent double cuticle in *C. vivipara* in contrast with the single cuticle in *B. saprophaga*, and the cuticular pores present in the former are apparently lacking in the latter. The gubernaculum in *C. vivipara* is polygonal in shape in contrast with the arcuate gubernaculum in *B. saprophaga*, and the tail is gradually attenuated to the tip in the former in contrast with the narrow tapering tail carried on a conical terminus in the latter. Lastly, the reproductive habit in the two forms is different; *C. vivipara* is ovoviviparous and *B. saprophaga* is oviparous. The stomatorhabdions in the two genera differ to a certain degree but the difference here is more interpretational rather than structural except for the anteriormost rhabdion, which differs in the degree of sclerotization in the two forms.

Acknowledgment

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STOMACH CONTENTS AND MICROFEEDING OF SOME COMMON CIRRIPEDES[1]

H. BARNES[2]

Abstract

An account is given of the stomach contents of some common barnacles; these include both intertidal and sublittoral species. The contents reflect to some extent the environment, species taken from the same place at the same time having similar contents. With some species, a particle size analysis was possible on a pooled sample of stomach contents. It is evident that much debris is present, and that particulate matter with a diameter less than 2 μ can be taken. Detritus may be an important source of nutriment with sublittoral species. Small particles cannot be taken by the captorial activity of the cirri. It is considered that glandular secretions at the base of the cirri, where there are numerous setae, enmesh the food, which is then carried forward to the mouth parts to be rejected or accepted.

Introduction

Relatively little is known of the food of barnacles. The musculature and movements of the cirri were described by Darwin (3), who pointed out how well they are adapted to a captorial function. A detailed account of captorial feeding in *Pollicipes polymerus* J. B. Sowerby, a stalked species which does not have a regular cirral beat, has been given by Barnes and Reese (2). Southward (7) has, however, shown that much smaller particles are removed from suspension than can be taken by the captorial action of the cirri. It is known that the co-ordinated movements of the cirri and body, or even of the latter alone, cause a current of water to flow over the mouth parts and through the mantle cavity to give rise to an exhalent current over the back of the cirral net; this current serves a respiratory function and in it the hatched nauplii are ejected into the surrounding water (Barnes (1); Southward and Crisp (8), q.v. for further details regarding cirral activity). In order to obtain further information, the stomach contents of a number of species from different regions and habitats have been examined.

The Material and Methods

The animals were examined either immediately after collection or, when this was not possible, were fixed without delay. The body of the animal was removed from its shell, washed to remove any adherent matter from the mantle cavity, and then the stomach carefully dissected out. The stomach contents were removed and examined under both low and high power magnification; measurements were made of the more conspicuous objects. With

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two of the larger species it was possible to collect enough material to do a particle size analysis. For this purpose the pooled stomach contents from a large number of animals were shaken vigorously with water for several minutes; no dispersing agent was added. The suspension was then poured into a small settling cylinder. At the time appropriate to the selected particle size settling rate, the liquid was removed from above the appropriate depth; this was accomplished by a small-bore siphon tube held permanently in position, the level required being obtained by adjusting the height of the cylinder. This method was found more convenient than the conventional technique when dealing with small volumes of suspension. The following size groups were separated: less than 1.0 μ, 1–2 μ, 2–4 μ, 4–8 μ, 8–16 μ and greater than 16 μ diameter, the settling being repeated until the liquid above the appropriate level was clear. The fractions were dried at 105° C, weighed, and the loss on ignition determined; this loss is inorganic material some of which—but only a small fraction—originates in skeletal material. Total nitrogen content of the dry residue from a separate sample was determined for two of the species, using a standard semimicro-Kjeldahl technique.

Results

Three species common at Millport, Scotland, were examined, namely *Balanus balanoides* (L.), *B. crenatus* Brug., and *B. balanus* (L.) da Costa. The first is strictly intertidal and the next two, although found at extreme low water, are largely sublittoral species. The material was collected in the autumn.

B. balanoides

Only a few sand grains; some smaller sand particles; small pieces of detached algae; very few diatoms; larval echinoderms; small gastropod (120 μ), crustacean remains fairly common; gregarines were present.

B. crenatus

A few small sand grains; diatoms occasional; some crustacean remains; considerable amount of coagulated unrecognizable debris.

B. balanus

Although examined immediately after collection, the stomachs of many of the animals were almost empty. Occasional sand particles were present; a few diatoms; crustacean limbs fairly common; some unrecognizable debris; gregarines.

Five common species of the American west coast were examined; two, *B. cariosus* (Pallas) and *Tetraclita squamosa rubescens* Darwin (Pilsbry (6)), are common in the intertidal of regions with moderate or high wave action, both tending to occur in the lower intertidal below *Balanus glandula* Darwin. Indeed *B. cariosus*, a northern form (Alaska to northern California), is replaced by *Tetraclita squamosa* on the main Californian coast (Pilsbry (6),

Henry (5)). Two species, *Balanus rostratus alaskensis* Pilsbry and *B. nubilus* Darwin, are largely sublittoral, the former being found from Alaska to the Puget Sound region and the latter from British Columbia to Lower California. All these species grow to a large size, individuals with a basal diameter of 50 mm being common and in two species, *B. cariosus* and *B. nubilus*, a particle size analysis was made on the stomach contents. *Pollicipes polymerus*, an intertidal pedunculate, which is normally restricted to the mid and lower tide regions, was also examined. It ranges widely from Alaska to Southern California. All the material was collected during the summer.

B. cariosus

Sand grains, many of a large size (790×560 μ), were common; peridinians and diatoms of various types common (10 μ diameter centric diatoms); filaments of detached algae and fibrous organic debris; a number of gastropod eggs and small gastropods; limbs and other parts of crustaceans common (up to 30 μ); small copepods quite common; cirripede nauplii and cyprids frequent; certain amount of unrecognizable debris; no gregarines. The results of the particle size analysis were as follows:

Size group	% dry weight	% inorganic
< 1μ	5.2	40
1–2	4.6	48
2–4	2.9	47
4–8	73.9	6
8–16	3.7	49
> 16	9.7	68

The particulate matter of quite a large part of the stomach contents is concentrated in the 4–8 μ size group, which has a very low ash content; this material is doubtless largely crustacean remains. The remaining size groups are distributed evenly, each with a considerable inorganic content. The high inorganic content of the largest size group (>16 μ) is consistent with it being composed largely of sand. Some 10% of the material is less than 2 μ. The relatively high inorganic content is consistent with the total nitrogen content of 1.1%, a value too low for purely animal or plant remains.

Tetraclita squamosa rubescens

Sand particles were numerous, many of large size (200–300 μ); very few protozoans or diatoms; occasional filament of detached weed; small gastropods (200 μ) and bivalves (220×240 μ) common; by far the greatest part consisted of small crustacea or crustacean remains; cirripede nauplii and cyprids common; no gregarines; impression of very little fine particulate debris.

Balanus rostratus alaskensis

Small sand grains common (50–60 μ); peridinians and foraminifera common (30 μ); all types of diatoms common (centric diatoms up to 40 μ diameter); few diatom frustules complete, the greater number broken up; gastropod eggs; relatively few animal remains; some unrecognizable debris; no gregarines; there is apparently more small particulate matter than in previous species.

Balanus nubilus

Small sand particles common, a few up to 60 μ, most 10 μ; peridinians and foraminifera common; diatoms of all types common, the greater number broken into fragments; detached algal filaments both green and red; occasional gastropod eggs; animal remains infrequent; much fine particulate unrecognizable debris; gregarines very common. The results of a particle size analysis were as follows:

Size group	% dry weight	% inorganic
< 1μ	22.8	58
1–2	15.5	47
2–4	14.3	60
4–8	13.9	58
8–16	21.4	50
> 16	12.1	59

The material is fairly evenly divided between the size groups all of which have a similar and high inorganic content. There is a sharp contrast with *B. cariosus*, particularly in the absence of a proportion with low inorganic content and in the much higher proportion of the particles below 2 μ; in *B. nubilus* this forms almost 40% of the total.

Pollicipes polymerus

In most of the material only a few sand particles were present; very few diatoms present; there was only a small amount of fine particulate debris; by far the largest part consisted of crustacean remains, some of these being 500–1000 μ long; small copepods were common; cirripede nauplii and cyprids common.

Discussion

It is to be expected that the stomach contents will reflect the material available in the surrounding water, since the captorial activity per se appears to be non-selective; there is no evidence that material striking the cirri is rejected during the course of the cirral beat. This is reflected in the above results by the great similarity in the material from *Balanus rostratus* and *B. nubilus*, where the material was collected at the same time and in adjacent localities. Inorganic debris, diatoms, and animal plankton are all taken by the captorial activity, transferred to the mouth parts, and carried into the oesophagus. The size range taken in this way must be largely a function of the size of the filtering surface that the cirral net presents to the water, although it must be stressed that the close apposition and interlocking of the setae as the cirri retract changes continuously during the cirral stroke. The relative absence of sand in *Pollicipes polymerus* is, therefore, striking. It has been shown that in this species in the absence of a rythmical cirral beat the cirri are individually retracted in response to touch (Barnes and Reese (2)); it would appear that this reaction is not readily elicited by inorganic material. Experimental work (unpublished) has shown that the response to sand grains is variable and it would seem that the extra stimulus given by a struggling prey such as a copepod is most effective; there is little evidence that any chemotactic sense is involved at least as far as the cirri themselves

are concerned. If sand grains are continually carried to the mouth parts, however, there may be inhibition of the cirral response arising from irritation by inorganic material. On the other hand, this species has a well-defined rejection mechanism but this usually removes only finer material.

It is evident that quite small particles can be taken and that finely divided particulate matter may in some species form an important part of the diet. This seems to be particularly the case in sublittoral species; with intertidal species small crustaceans form an important part of the diet together with diatoms and diatom chains. At the time of the spring diatom increase, diatoms form a very conspicuous part of the food of *Balanus balanoides*. The contrast between the intertidal *B. cariosus* and sublittoral *B. nubilus* is very marked in this respect. However, perhaps the contrast is over-emphasized by examining large *B. nubilus*. Observations suggest that the very large individuals of this species are somewhat sluggish and rarely show a typical cirral beat. The fact that small particles of debris can be taken is of importance to deep-water species in which this material is far more available than diatoms or small crustaceans; dissection of deep-water species rarely shows remains that can be recognized.

It is now possible to enquire as to the mechanism by which these small particles are removed from the water, whether the cirri are executing their normal beat or whether only microfeeding is taking place. As has already been pointed out the body movements will cause a current of water to flow over the body and the mouth parts. Now, as Gruvel (4) first showed, the labrum and outer maxillae are well supplied with unicellular glands: he considered that food particles wiped off the cirri were cemented together by the secretions of these glands to form a 'bol alimentaire' which was then swallowed. Similar large glands also open near the base of the first, second, or third thoracic appendages and Thomas (9) thought that these were too far from the mouth to function in the manner suggested by Gruvel. He postulated that the second series of glands, termed by him suboesophageal tegumental glands, were used to collect undesirable particles and form a rejection mass. The truth would appear to lie between these two hypotheses. It has been shown (Barnes and Reese (2)) that in *Pollicipes polymerus* large particles are wiped off the cirri (although no cirral beat is involved) and are carried forward to the mouth; if the food material is 'unpalatable' it may be carried forward over the mouth parts (which under these conditions remain inactive) and rejected. It is suggested that in microfeeding, small particles are held amongst the abundant fine setae in the secretion from the glands, coagulated, and carried forward in exactly the same way, that is, largely by the reciprocating action of the first cirri. Whether the particles are then taken into the food tract will depend upon their nature, but the mechanism is exactly the same as for larger particles such as whole crustaceans; indeed the latter will in part be covered by any secretion. We have no knowledge of the stimulus deciding acceptance or rejection, but it is difficult to avoid the implication that a chemotactic response is involved.

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THE NAUPLIAR STAGES OF BALANUS HESPERIUS PILSBRY[1]

H. BARNES AND MARGARET BARNES[2]

Abstract

The details of morphology of the six naupliar stages of *Balanus hesperius* are described, the material being referable to the forma *laevidomus* of Pilsbry. The nauplii are particularly characterized by a long and somewhat slender body and rather stiff appendages, the rami of which are thickened proximally. The general pattern of development conforms to that of other nauplii and is remarkably similar in all balanids. Tables of sizes and setation are given.

Introduction

Pilsbry (5) first described *Balanus hesperius* from Alaskan waters. He further separated, although with some hesitation, a new subspecies, *B. hesperius nipponensis*, from Japan, and a new form, *B. hesperius laevidomus*, which occurs southwards from Alaska to Monterey, California (Henry (3)). This form *laevidomus* is common on shells dredged near Friday Harbor, Washington, and all the material used in this study is referable to this form without, however, any implications with regard to its systematic status.

The Material and Methods

As has been described (1) for *B. nubilus* the second and third naupliar stages were raised in culture from stage I nauplii that had been hatched from ripe egg masses carefully removed from the mantle cavity of adults. The appearance of the nauplii is so distinctive that there is then no difficulty in recognizing the later stages in plankton collections. The larvae are fairly common in such collections taken during the summer in the waters adjacent to Friday Harbor, San Juan Archipelago, Washington. The cypris stage was not determined. With the exception of stage I, all measurements were made on formalin-preserved planktonic material collected with a fine net. The total length was measured from the frontal margin to the tip of the caudal spine and the carapace length from the former to the posterior border of the carapace. The carapace width was taken as the greatest width of the body behind the frontolateral horns. All measurements were made with the larvae lying in a natural position with the ventral side uppermost.

Results

General Features of the Nauplii

Six naupliar stages were found; outline drawings are given in Fig. 1 and the various dimensions set out in Table I. The larvae are large compared with

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TABLE I

Sizes (μ) of larval stages of *Balanus hesperius laevidomus*

Stage	Total length	Carapace			Frontolateral horns
		Width	Length	Spines	
I	265	129	–	–	–
II	468	153	–	–	77
III	574	200	–	–	93
IV	704	243	368	114	140
V	867	338	483	180	169
VI	1080	473	644	288	222

those of other species but, because they are relatively slender, never give the bulky impression of the nauplii of *B. nubilus* and *B. balanoides*. In the living animal the appendages appear to be held more rigidly than in other species and to beat more slowly—features which are common to all the stages. The frontal margin, except in stage I, is almost straight and the frontolateral horns and carapace spines, when present (stages IV–VI), are well developed. The abdominal process is somewhat shorter than the caudal spine, the latter tending to curve a little to the dorsal side and so appearing foreshortened when the animal is viewed from the ventral side; this curvature varies amongst the individuals.

In stage I (Fig. 1) the body is more or less pear-shaped with the frontolateral horns closely appressed to the sides of the carapace; in preserved

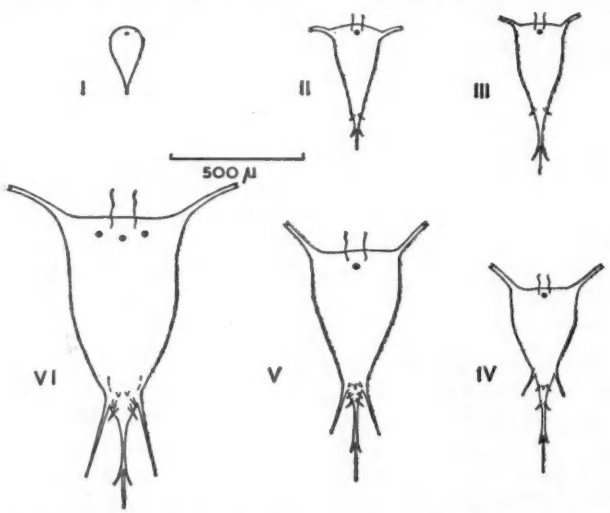

FIG. 1. Outline drawings of the six naupliar stages of *Balanus hesperius laevidomus* showing shape and the more important spinous processes.

material, there is little to distinguish it in general outline from many other species, although once known its somewhat more elongated appearance is evident. Stage I is rarely taken in the plankton. Frontal filaments are absent; in this and all succeeding stages a median eye is present.

In stage II the frontolateral horns are well developed; they are slightly curved and project laterally. Frontal filaments are present in this and all

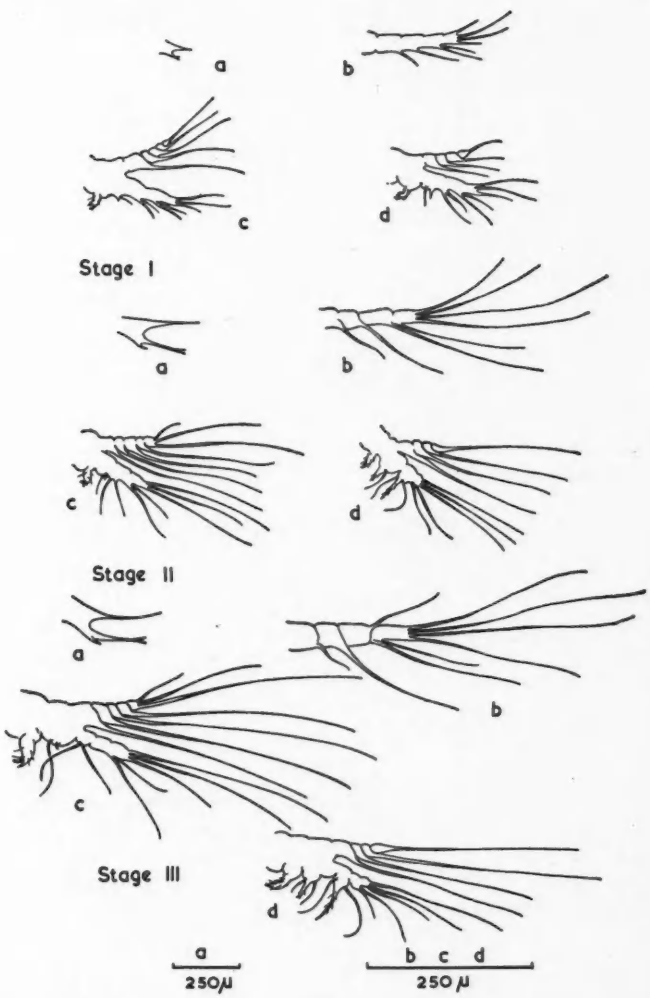

FIG. 2. Stages I, II, and III larvae of *Balanus hesperius laevidomus*: (*a*) lateral view of abdominal and caudal processes, (*b*) antennule, (*c*) antenna, (*d*) mandible.

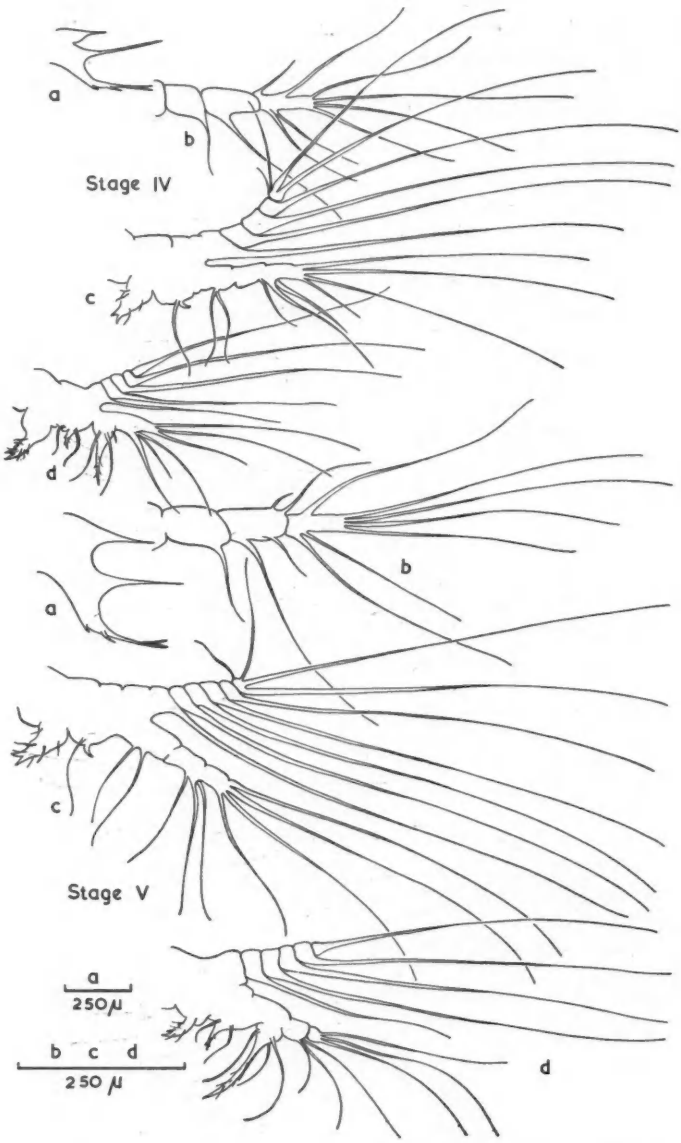

FIG. 3. Stages IV and V larvae of *Balanus hesperius laevidomus*: (*a*), (*b*), (*c*), and (*d*) as in Fig. 2.

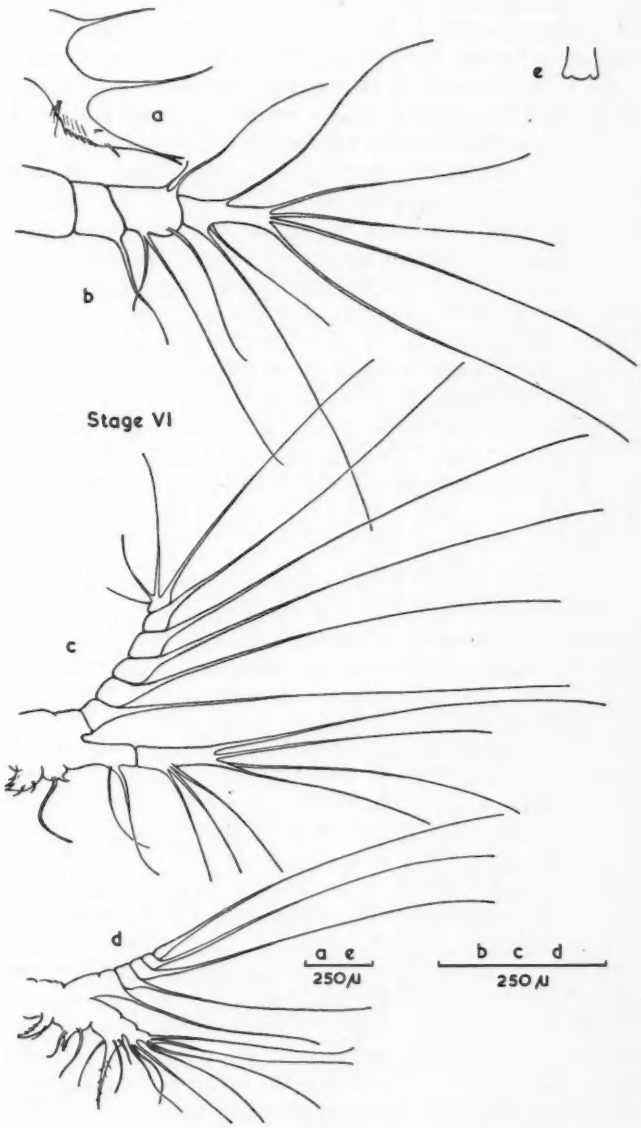

FIG. 4. Stage VI larva of *Balanus hesperius laevidomus*: (*a*), (*b*), (*c*), and (*d*) as in Fig. 2; (*e*) labrum.

subsequent stages. The lateral margins of the carapace slope gradually towards the mid-line so that, viewed from the ventral side, the nauplius has a distinctly triangular outline. The abdominal process, which approaches in length the caudal spine, bears a pair of well-defined spines; at its posterior extremity is a furca, whose rami are slender and often slightly curved.

In stage III the frontolateral horns, which are now prominent, project more to the anterior. The anterior parts of the lateral margins of the carapace are at first straight but to the posterior gradually approach the mid-line; the outline is, therefore, less regularly triangular. The abdominal process still bears a single pair of spines; the antennule has a preaxial seta.

Stage IV has the posterior border of the carapace clearly delimited and it bears a pair of long tapering carapace spines present in all subsequent stages. Stage V resembles stage IV in outline but the carapace spines are stronger and about equal in length to the frontolateral horns. A further pair of abdominal spines are present in a position between the first and second series.

Stage VI although large still retains a somewhat delicate appearance. The caudal spines are now longer than the frontolateral horns. The abdominal process is more swollen and the segmented cirriform appendages can be seen underlying the exoskeleton. A pair of lateral eyes, usually well pigmented, are present.

The Carapace

A distinct posterior border to the carapace with a pair of spines arising from it first appears in stage IV. This further confirms the statement of Jones and Crisp (4) that only at this stage do these features develop in balanid nauplii. The spines are well developed in stages IV–VI.

Abdominal Process and Caudal Spine

The abdominal process, resembling that of other balanid nauplii, arises in a mid-ventral position posterior to the labrum and projects ventrally and posteriorly. In the bulbous proximal portion the cirral appendages develop and proximally the process forms a long stem with a terminal furca. Raised areas bearing long fine hairs mark the part where the maxillules develop. The arrangement of the spines on the process follows the pattern found in other species: a single pair is found in stages II and III; in stage IV a further pair, with a median spine between them, is added towards the anterior; in stage V an additional pair of spines is found between the first two series. The median spine of the second series is lost in stage VI. It is in stage VI that a series of spines which contain the tips of the developing cirri are found on the bulbous portion of the process and the differentiation of the associated muscle blocks can be clearly seen. Posteriorly the furca of the abdominal process extends more than two-thirds the distance to the tip of the caudal spine.

The Labrum

This is trilobed, all lobes being setose. The large median lobe hardly projects posteriorly beyond the level of the smaller lateral lobes.

The Eyes

In all stages a median eye is present. Stage VI has, in addition, paired lateral eyes which are usually quite deeply pigmented. With the animal lying on its dorsal side these lateral compound eyes have the appearance of being set slightly anterior to the median eye.

Setation of the Appendages

Paired antennules, antennae, and mandibles are present in all stages. Stages II–VI have a pair of frontal filaments (stylets) arising on either side of the median eye. Figures 2–4 show the setation of the appendages; adopting Bassindale's (2) notation, which is now conventional, we have set out their form in Table II.

TABLE II

Setation formulae for the naupliar stages of *Balanus hesperius laevidomus* (all notations according to those given by Bassindale (2))

Stage	Antennule	Antenna	Mandible
I	0.4.2.1.1.	0.1.4.–0.3.2.2.2.G.	0.1.3.–0.3.2.2.2.G.
II	0.4.2.1.1.	0.3.4.–0.3.2.2.2.G.	0.1.3.–0.3.2.3.2.G.
III	1.4.2.1.1.	0.3.4.–0.3.2.2.2.G.	0.1.3.–0.3.2.3.3.G.
IV	1.1.4.2.1.1.	0.4.4.–0.4.3.2.2.G.	0.1.4.–0.3.3.4.3.G.
V	1.1.1.4.2.1.1.1.	0.4.5.–0.4.3.2.2.G.	0.1.4.–0.3.3.4.3.G.
VI	1.1.1.4.2.1.2.1.	0.4.5.–0.4.3.2.2.G.	0.1.4.–0.4.3.4.3.G.

The antennule conforms to the pattern of other species, with a preaxial seta first appearing in stage III. Further preaxial setae are added in stages IV and V and additional postaxial setae in stages V and VI. The setation appears to have no specific significance, although in general the setae are fewer in number than in other species that have been described; in stage VI, for example, the total number of setae on the antenna and mandible are 20 and 19 respectively, whereas 26 and 21 are not uncommonly found. Furthermore, only two postaxial setae are present in the third group of the antenna in all stages and one of these remains throughout development as a small stump; in other species one seta of this group usually takes the form of a very distinct and stout spine. The gnathobase of the mandible bears a single stout pointed process which is set with stiff setules, whereas that of the antenna, also with a complex series of setules, is markedly bifid. The most marked feature of the appendages is their great length and the rod-like character of the distal part.

No variation of setation between individuals was observed.

Discussion

The large size, delicate appearance, distinctive shape, and apparent rigidity of the appendages with their more deliberate beat make it easy to recognize the species in west coast plankton; there is little danger of confusing it with

other species that have been seen in summer plankton collections. The stages may be largely separated on size and general appearance but other characters which do not require dissection may also be used. Stage II has its triangular shape; stage III, less triangular, has a preaxial seta on the antennule. In stage IV the posterior border of the carapace is clearly defined and bears the pair of long carapace spines. Stage V has an extra pair of spines on the abdominal process not present in the previous stage. Stage VI, a somewhat more bulky nauplius with the paired and pigmented lateral eyes, the swollen abdominal process with developing cirriform appendages, is easily distinguished.

The results serve to confirm recent work on cirripede nauplii, which tends to emphasize how uniform is the development in the group. No reliable differences in setation have been found in the antennules of any species and, while the setation of the antennae and mandibles vary from species to species, there is little indication that it is of any specific importance. A posterior border to the carapace and carapace spines appear in stage IV, and the series of abdominal spines follow the same pattern from one stage to the next, as has been found in other species.

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PARASITES FROM ELASMOBRANCH HOSTS FROM THE MAGDALEN ISLANDS REGION OF THE GULF OF ST. LAWRENCE[1]

BETTY J. MYERS

Abstract

Of 173 skates and 157 sharks examined from the Magdalen Islands region of the Gulf of St. Lawrence, 91 and 30, respectively, were infected with helminth parasites. The parasites found are recorded.

During the summer of 1955 various species of skates and sharks taken by net and long-line fishing in the Magdalen Islands region of the Gulf of St. Lawrence were examined for parasites.

Cestodes and trematodes were stained with Ehrlich's acid haematoxylin, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were cleared in lactophenol and examined in temporary mounts of the same media.

The parasites found during this survey are listed below:

Raja laevis Mitchill, 1817 (synonym *Raja stabuliforis* Garman, 1913) (barndoor skate)

Of 29 specimens examined 15 were parasitized.

CESTODES

Acanthobothrium coronatum (Rud., 1819) van Ben., 1850
Grillotia erinaceus (van Ben., 1858) Fuhrmann, 1931
Phyllobothrium dagnalli Southwell, 1927
Phyllobothrium sp.

TREMATODES

Hemiurus levinseni Odhner, 1905 (pseudoparasite)
Steganoderma formosum Stafford, 1904 (pseudoparasite)

NEMATODES

Anisakis type larvae
Eustoma rotundatum (Rud., 1819) Punt, 1941

Raja ocellata Mitchill, 1815 (synonym *Raja diaphanes* Garman, 1913) (spotted skate, winter skate, eyed skate, big skate)

Of 62 specimens examined 28 were parasitized.

CESTODES

Acanthobothrium coronatum (Rud., 1819) van Ben., 1850
Anthobothrium cornucopia van Beneden, 1850
Anthobothrium variabile (Linton, 1889) Wardle and McLeod, 1952
Echeneibothrium variabile van Beneden, 1850
Grillotia erinaceus (van Ben., 1858) Fuhrmann, 1931

[1]Manuscript received December 10, 1958.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

Phyllobothrium sp.
TREMATODES
Otodistomum veliporum (Creplin, 1837) Stafford, 1904
Podocotyle atomon (Rud., 1802) Odhner, 1905 (pseudoparasite)
NEMATODES
Anisakis type larvae
Porrocaecum type larvae
Eustoma rotundatum (Rud., 1819) Punt, 1941

Raja radiata Donovan, 1807 (thorny skate, starry skate)
Of 82 specimens examined 48 were parasitized.
CESTODES
Acanthobothrium coronatum (Rud., 1819) van Ben., 1850
Anthobothrium cornucopia van Beneden, 1850
Anthobothrium variabie (Linton, 1899) Wardle and McLeod, 1952
Echeneibothrium variabile van Beneden, 1850
Phyllobothrium sp.
TREMATODES
Otodistomum veliporum (Creplin, 1837) Stafford, 1904
NEMATODES
Anisakis type larvae
Eustoma rotundatum (Rud., 1819) Punt, 1941

Lamna nasus (Bonnaterre, 1788) (synonym *Isurus punctatus* Garman, 1911) (mackerel shark, porbeagle, blue dog)
Of three specimens examined two were parasitized.
CESTODES
Dinobothrium sp.
Phyllobothrium dagnalli Southwell, 1927

Squalus acanthias Linnaeus, 1758 (spiny dogfish, dogfish, piked dogfish, gray fish)
Of 154 specimens examined 28 were parasitized.
CESTODES
Phyllobothrium sp.
TREMATODES
Hemiurus levinseni Odhner, 1905 (pseudoparasite)
Otodistomum veliporum (Creplin, 1837) Stafford, 1904
Prosorhynchus squamatus Odhner, 1905 (pseudoparasite)
Steganoderma formosum Stafford, 1904 (pseudoparasite)
NEMATODES
Anisakis type larvae

THE EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF THE FREE-LIVING STAGES OF DOCHMOIDES STENOCEPHALA (RAILLIET, 1884) (ANCYLOSTOMIDAE : NEMATODA)[1]

H. C. GIBBS[2] AND K. E. GIBBS[3]

Abstract

The effects of temperature on the development of the free-living stages of *Dochmoides stenocephala* (Railliet, 1884) have been studied using agar cultures. Development was obtained at as low a temperature as 7.5° C. The optimum temperature for the free-living stages was considered to be 20° C. The time of development of the larvae to the infective stage was inversely proportional to the ambient temperature between the limits of 7.5° C and 27° C.

Introduction

Dochmoides stenocephala, the northern carnivore hookworm, has a life cycle typical of the hookworms, in that part of it is spent externally as ova and free-living larvae. It has been reported from as far north as Craig Harbour, Ellesmere Island, by Cameron *et al.* (1) and has a wide distribution throughout the Canadian north. This suggests that the free-living stages of this worm possess characteristics which enable them to resist the highly variable and often rigorous climatic conditions prevalent in this area. It was therefore decided to examine the effects of temperature on the development of the free-living stages from the ovum to the infective larval stage.

The factors influencing the development of the free-living stages of the hookworms have long been subjects of investigation. Practically all of these studies were made on faecal cultures. While faecal cultures are convenient and easy to use, their complex and variable nature, as was pointed out by McCoy (4), has always been a complicating factor in the interpretation of results. The work of Stoll (5) on various culture media for hookworms provides a good example. The most consistent results were obtained using humus cultures but even with this substance the coefficient of variation for the number of larvae recovered was 36% in one series while in another it ranged up to 134%.

McCoy (4) described a method for obtaining hookworm ova free from faeces and bacteriologically sterile. He found that these ova would hatch and larvae develop to the infective stage on agar cultures of living bacteria. The coefficient of variation that he obtained was 9%. Furthermore, under favorable conditions of temperature, about 90% of the larvae reached the infective stage on cultures of a suitable bacterium.

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Materials and Methods

The culture method used was a modification of that devised by McCoy (4) for studying the effects of temperature on the development of the free-living stages of *Ancylostoma caninum*.

Fresh faeces were collected each morning from infected dogs. Ova were collected by first adding an excess of cold tap water to the faeces in large beakers, stirring well, and then sieving the mixture through a series of strainers of decreasing coarseness to remove large particles. The strained mixture was allowed to sediment for about 45 minutes in a refrigerator, the supernatant decanted, and more water added. The process was repeated until the supernatant became clear. At this point, after decanting, the sediment was diluted by one-half with cold, saturated sucrose solution. The mixture was poured into flat glass dishes with ground edges until they were filled. Sheets of plate glass were then placed over the pans in contact with the fluid. After 30 minutes the plate glass was carefully lifted and the adhering ova washed into a beaker. The ova were rinsed in distilled water, concentrated, and counted. After they were counted they were centrifuged down and the excess water removed. The ova were sterilized in 5% antiformin solution for 15 minutes. After sterilization they were rinsed three times in sterile distilled water and were then ready for use. Care was taken throughout to ensure that all solutions used were not above 5° C.

The agar medium was prepared using the following formula:

14.4 g nutrient agar,*
2.4 g nutrient broth base,*
2400 ml distilled water.

The agar mixture was poured in 80-ml portions into 250-ml Erlenmeyer flasks, stoppered with cotton plugs, and autoclaved for 15 minutes.

The agar was inoculated with saline suspensions of pure cultures of *Escherichia coli* obtained from the Department of Bacteriology, Macdonald College. After incubation of the flasks at 37° C for 48 hours, a 1-ml suspension of sterile ova was added to each and they were then placed in incubators at the desired temperatures. Simultaneously, a small Petri dish containing an aqueous suspension of ova was incubated, and used to check the time of hatching.

Control cultures for each series of cultures were held at 20° C to check the viability of the ova.

To estimate the rate of larval development, individual cultures were removed from the incubators at specific intervals. Fifty milliliters of water was added to each culture, swirled carefully so as not to break the agar, and decanted into a beaker. Four 0.5-ml portions of this decanted liquid were then removed and the living and dead larvae in each were counted. Twenty-five living larvae were then killed by gentle heating and measured using an ocular micrometer. The values obtained were averaged and the mean value was taken as the average size of the larvae at the time of examination.

*Difco laboratories.

Results

Development at 5° C (Table I, Fig. 1)

This was the lowest temperature at which development was studied. The ova embryonated slowly and began hatching after the 3rd day. Hatching was slow and it was not until the 23rd day that most of the ova had hatched. The first-stage larvae averaged 0.304 mm on hatching, were very sluggish, and grew slowly. After 23 days they averaged 0.369 mm in length, the longest ones being 0.437 mm and the shortest 0.347 mm. Thereafter they began dying off in large numbers and in these experiments none of the observed larvae reached the infective stage.

TABLE I

Development of ova and larvae at 5.0° C and 7.5° C

Temperature	Age (days)	Length (mm)		Estimated total larvae	% alive	Infective larvae present	Culture series
		Av	Range				
5.0° C	2.5	0.304		100	100.0	—	2
	6	0.338	0.304–0.399	325	100.0	—	2
	9	0.338	0.304–0.361	250	100.0	—	2
	15	0.327	0.304–0.418	575	100.0	—	2
	21	0.389	0.304–0.437	400	50.0	—	3
	23	0.369	0.342–0.437	850	64.7	—	3
	28	0.418	0.304–0.437	700	20.0	—	3
7.5° C	1.5	0.304		150	100.0	—	3
	3	0.338	0.304–0.342	350	100.0	—	3
	8	0.360	0.304–0.418	1200	88.0	—	3
	14	0.418	0.342–0.513	1700	62.3	—	3
	21	0.532	0.418–0.608	1680	35.0	+	3
	28	0.565	0.456–0.608	1600	30.0	+	3

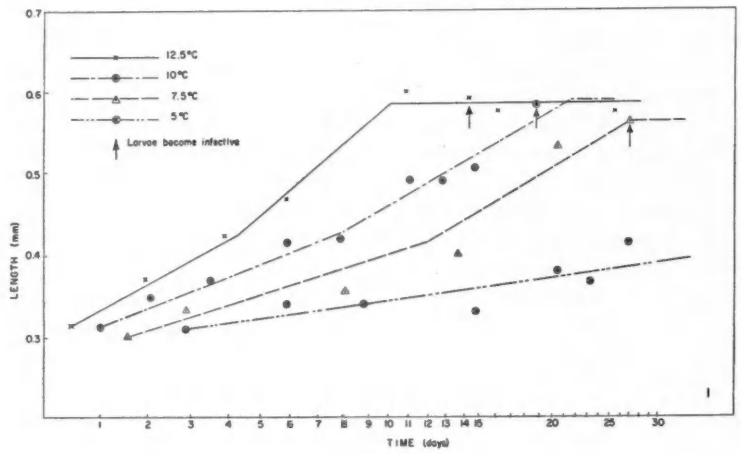
Development at 7.5° C (Table I, Fig. 1)

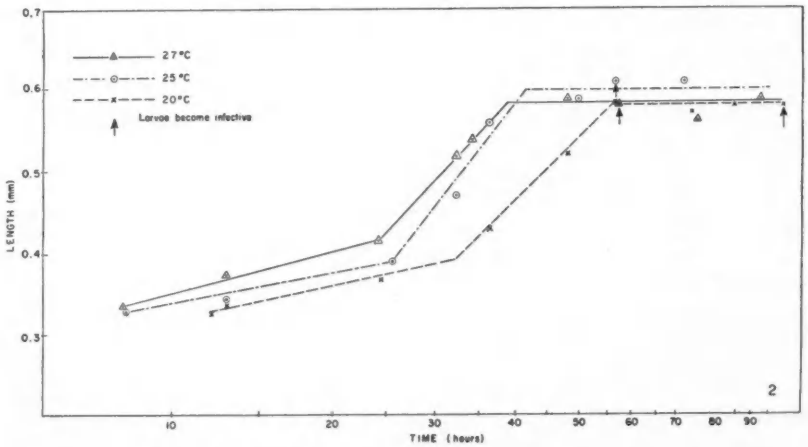
At this temperature a few larvae had hatched after 36 hours of incubation, but it was not until after 8 days that most appeared to have hatched. They grew slowly but by 28 days some were as long as 0.608 mm and a few had become infective. After 13 days those larvae which were still in the first and second stages began to die off. This was the lowest temperature at which development took place in these experiments. An interesting observation made at 7.5° C was that when apparently full-grown second-stage larvae were removed and warmed to 25° C, they quickly went into lethargus and within 6 hours after removal they had reached the infective stage.

The average final length of the larvae at this temperature was slightly less than at higher temperatures.

Development at 10° C (Table II, Fig. 1)

A few ova hatched as early as 24 hours after the start of the experiment, but it was not until after 6 days had elapsed that the majority had hatched.





FIGS. 1 and 2. The effect of temperature on the free-living development of *D. stenocephala*.

Development was slow but after 21 days a number of larvae had reached the infective stage. However, after 13 days some of the non-infective larvae began dying off and by 20 days only about 30% were still alive, these being almost all infective. At this time it was also observed that a few ovic larvae were viable and active. The average length of the infective larvae was 0.581 mm at 21 days. At 13 days it was observed that although some individuals were as long as 0.608 mm some were only 0.304 mm. At this point the smaller ones began dying off and this increased the value of the average size of the survivors.

TABLE II

Development of ova and larvae at 10.0° C and 12.5° C

Temperature	Age (days)	Length (mm)		Estimated total larvae	% alive	Infective larvae present	Culture series
		Av.	Range				
10.0° C	1	0.312	0.304–0.320	350	100.0	—	1
	2	0.350	0.304–0.380	250	100.0	—	1
	4	0.372	0.304–0.456	375	100.0	—	1
	6	0.420	0.304–0.570	2475	99.0	—	1
	8	0.423	0.323–0.475	1700	100.0	—	3
	11	0.481	0.380–0.608	3000	100.0	—	1
	13	0.490	0.304–0.608	1225	45.5	—	3
	15	0.509	0.456–0.551	3175	74.0	—	1
	20	0.581	0.532–0.627	1725	29.0	+	3
12.5° C	1	0.311		275	100.0	—	1
	2	0.372	0.304–0.418	650	100.0	—	1
	4	0.422	0.380–0.456	2300	100.0	—	1
	6	0.471	0.418–0.570	1850	100.0	—	1
	11	0.600	0.532–0.646	1900	100.0	—	1
	15	0.580	0.551–0.646	2500	99.0	+	1
	18	0.566	0.494–0.608	1905	69.6	+	1
	26	0.566	0.532–0.608	1840	98.4	+	1

Development at 12.5° C (Table II, Fig. 1)

Development was slightly faster at this temperature than at 10° C. Some larvae had hatched after 12 hours and after 4 days most had hatched. Some larvae reached the infective stage after 15 days, with an average size at this time of 0.580 mm. There was less variation in size between individual larvae at this temperature than at lower temperatures, and development although slow was uniform. This temperature was not unfavorable for larval development and survival, as after 26 days of incubation there were still 98.4% of the larvae alive in the cultures.

Development at 20° C (Table III, Fig. 2)

At this temperature many ova had hatched after 12 hours; the average size was 0.327 mm. Development was rapid so that by 96 hours most larvae had reached the infective stage. Series of cultures were kept for up to 42 days to study the longevity of infective larvae at 20° C. After 35 days 86% were still alive but after this the larvae started to die off and there were only 35% alive after 42 days.

Development at 25° C (Table III, Fig. 2)

A few ova had hatched by 8 hours and the majority had hatched by 25 hours. The larvae grew rapidly and averaged 0.558 mm within 36 hours. By 57 hours there were infective larvae present. This is very rapid development. The maximum length obtained was 0.684 mm.

Although initially the growth of the first-stage larvae at 25° C was slower than that at 27° C, the final size attained by the larvae was greater and the

TABLE III

Development of ova and larvae at 20.0° C and 25.0° C

Temperature	Age*	Length (mm)		Estimated total larvae	% alive	Infective larvae present	Culture series
		Av.	Range				
20.0° C	12h	0.327	0.304–0.361	1000	100.0	—	2
	13h	0.338	0.304–0.380	350	100.0	—	3
	24h	0.370	0.342–0.381	1975	100.0	—	1
	36h	0.435	0.380–0.570	1125	100.0	—	3
	48h	0.521	0.418–0.570	2075	100.0	—	1
	56h	0.581	0.513–0.608	1100	75.0	+	3
	72h	0.570	0.513–0.608	1225	98.0	+	1
	84h	0.578	0.532–0.646	1700	83.3	+	2
	6d	0.578	0.456–0.608	2125	98.8	+	1
	13d	0.593	0.532–0.646	2150	100.0	+	1
	35d	0.608	0.570–0.646	1925	86.5	+	1
	42d	0.608	0.570–0.646	2050	38.0	+	1
25.0° C	8h	0.327	0.304–0.380	125	100.0	—	2
	13h	0.346	0.304–0.380	575	100.0	—	2
	25h	0.391	0.342–0.513	1050	100.0	—	2
	31h	0.463	0.342–0.608	1825	87.9	—	2
	36h	0.558	0.456–0.608	1875	97.1	—	2
	50h	0.590	0.532–0.646	1700	87.9	—	2
	57h	0.612	0.532–0.684	1725	89.7	—	2
	72h	0.608	0.570–0.684	1900	88.0	+	2
	13d	0.608	0.570–0.684	2075	3.0	+	3

*h=hours.
d=days.

TABLE IV

Development of ova and larvae at 27.0° C and 37.0° C

Temperature	Age*	Length (mm)		Estimated total larvae	% alive	Infective larvae present	Culture series
		Av.	Range				
27.0° C	8h	0.334	0.304–0.380	550	100.0	—	2
	13h	0.372	0.304–0.418	1750	100.0	—	2
	24h	0.414	0.342–0.532	1325	100.0	—	2
	32h	0.517	0.456–0.570	1550	100.0	—	3
	34h	0.536	0.399–0.608	1175	100.0	—	2
	48h	0.585	0.532–0.627	2050	100.0	+	2
	57h	0.578	0.532–0.608	2450	100.0	+	2
	75h	0.563	0.532–0.608	1600	75.4	+	2
	93h	0.581	0.551–0.608	2000	48.2	+	2
	10d	0.580	0.551–0.608	1700	5.0	+	2
	14d			1700	0	+	2
37.0° C	9h	0.369	0.304–0.418	375	100.0	—	1
	13h	0.369	0.304–0.418	475	100.0	—	1
	25h	0.365	0.304–0.494	450	71.4	—	1
	48h	0.463	0.361–0.532	450	39.6	—	1
	77h			375	0	—	1

*h=hours.
d=days.

rate of growth from the second to the third stage appeared to be faster. The time at which they reached this point was less than at 27° C. After 13 days at 25° C, most of the larvae were dead.

Development at 27° C (Table IV, Fig. 2)

Some ova had hatched after 8 hours and the majority had hatched by 13 hours. The larvae grew very rapidly, attaining the infective stage after about 51 hours, which is extremely rapid development. The maximum length attained by the larvae at this temperature was 0.646 mm, which was slightly less than at 25° C. At 27° C the infective larvae did not survive long and after 10 days most of the larvae had died.

Development at 37° C (Table IV)

At this temperature a number of ova had hatched after 9 hours but it was too high for them since after 77 hours no living larvae were found in the cultures. A few reached a maximum length of 0.532 mm.

Discussion of Results

Examination of the curves showing the rates of development of the larvae, in Figs. 1 and 2, shows that in each case it is a triphasic curve in which the first phase is represented by the time from hatching until the larvae reach a length of about 0.40–0.43 mm. The second phase is from this point until a maximum of 0.60 mm is reached. Then there is a levelling off with no increase in size in the third phase. The first phase represents the growth of the first-stage larva until it goes into lethargus, the second phase represents the growth of the second stage larva from molting until it goes into lethargus, and the third phase represents the molt of the second and attainment of the final or third stage.

The minimum and maximum temperatures at which the ova would hatch and larvae develop to the infective stage in these experiments were 7.5° C and 27° C respectively. Below 7.5° C some ova hatched after a long interval and there was partial larval development. However, none of the larvae reached the infective stage in this experiment at temperatures below 7.5° C. Above the maximum temperature, at 37° C, while the hatching of the ova was rapid and development of the larvae was initially rapid, the temperature proved to be excessive and all larvae quickly perished.

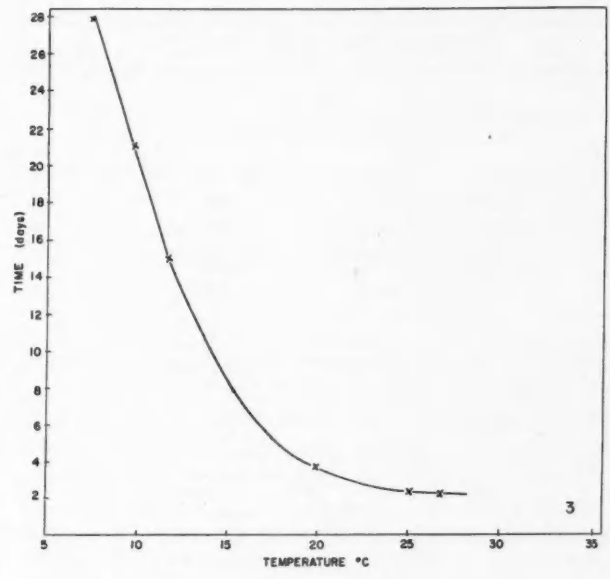
At 7.5° C, 21 to 28 days were required for the larvae to reach maturity, while at 27° C they became mature in 51 hours. The time of transformation of mature second-stage larvae to infective larvae tended to be very uniform at the higher temperatures, while at the lower ones it was less uniform and there was more variation seen in size at transformation. This discrepancy in rate of development characterized the growth of larvae at the lower temperatures (5° C, 10° C, and 12.5° C), while at the higher temperatures (20° C and 27° C) development was more uniform. Variability was especially evident in the hatching ability of the ova. At 7.5° C and 10° C active ovic larvae

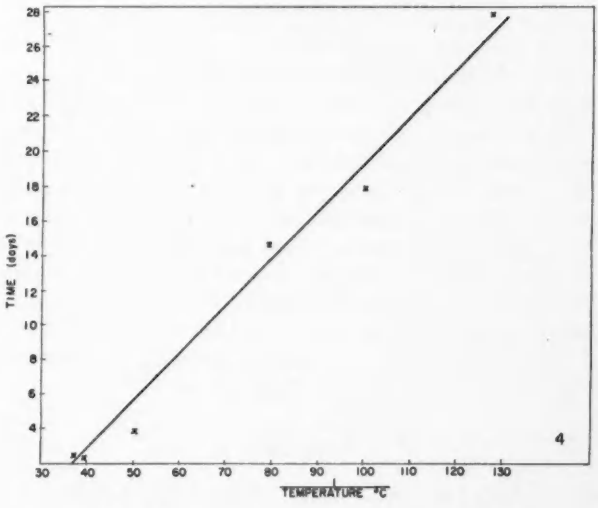
were still observed in cultures after 21 days while some of the already hatched larvae had reached the infective stage and the intermediates were dying off. It therefore appears that under suboptimal conditions for development a certain proportion of the larvae, more vigorous than the rest, proceeded straight through with their development. The less vigorous larvae either failed to hatch or developed slowly and died before attaining the infective stage. This phenomenon, to a lesser degree, was observed even in cultures at the most favorable temperatures. Since the conditions in these cultures appeared to be favorable for growth of the majority of individuals, there must be some inherent factor that prevents the development of these other individuals. This was also the experience of McCoy (4) with cultures of *A. caninum*. It must be concluded that there is a considerable range of variation in the ability of a given lot of larvae to adjust to environmental conditions, and also that the unhatched larvae and the infective larvae are more resistant to low temperatures than are the preinfective stages.

It is indeed remarkable to find that larvae will develop to the infective stage at as low a temperature as 7.5° C. Perroncito (reported by Looss (3)) is the only worker who claims to have reared infective hookworm larvae at as low a temperature as 12° C. McCoy (4) found that the ova of *A. caninum* would hatch at 12° C but would not develop to the infective stage. The lowest temperature at which *A. caninum* would develop to maturity was 15° C over a period of 17 to 22 days.

The determination of the optimum temperature for development rests on four factors: (1) rapidity of development to the infective stage, (2) maximum length attained by the infective larvae, (3) number of larvae which become mature, and (4) longevity of the infective larvae.

If we consider the first point, rapidity of development, we see that the optimum temperature must be either 25° C or 27° C. From Fig. 2 it will be seen that although development of the first-stage larvae is faster at 27° C, development of the second-stage larvae is faster at 25° C. Secondly, the average length of the larvae as well as the maximum length attained is greater at 25° C than at 27° C or 20° C. Thirdly, the number of larvae becoming mature appears to be about the same for the three temperatures. However, fourthly, the longevity of the infective larvae appears to be greater at 20° C than at 25° C or 27° C. This is an important point as it is imperative that an infective larva remains alive as long as possible in order to have the maximum opportunity for infecting its host. The optimum temperature for the free-living stages, from this experiment, therefore appears to be 20° C. It may be argued that under the conditions of this experiment bacterial growth would be increased at the higher temperatures, 25° C and 27° C, which would result in greater competition among the organisms for available oxygen at the surface of the cultures. This in turn might partly account for the death of the larvae. Law (2) felt that the optimum temperature for *D. stenocephala* was probably around 25° C. McCoy (4) found that for *A. caninum* the optimum temperature was 30° C and that larvae developed rapidly to maturity at





FIGS. 3 and 4. The relationship of the time of development of the infective larvae of *D. stenocephala* to the temperature (Fig. 3) and to the reciprocal of the temperature (Fig. 4).

TABLE V

Comparison of size, rate of development, and survival of larvae on reaching the infective stage at various temperatures

Temperatures (° C)	1/temperature	Size (mm)		% alive	Days to infective stage
		Average	Maximum		
7.5	0.133	0.532	0.608	35.0	28
10	0.100	0.581	0.627	29.0	21
12.5	0.080	0.566	0.646	69.6	15
20	0.050	0.578	0.646	83.3	4
25	0.040	0.608	0.684	88.0	2.4
27	0.037	0.563	0.646	75.0	2.4

37° C. *A. caninum* apparently requires higher temperatures than *D. stenocephala* for development. Herein, it is felt, lies part of the explanation of the adaptation of *D. stenocephala* to a subarctic and arctic environment. The ability to develop at low temperatures makes it possible for this nematode to extend the limits of the comparatively short "growing season" into the spring and into the fall.

An interesting observation was that the development of apparently fully grown second-stage larvae held at low temperatures could be rapidly accelerated if the larvae were placed in a warmer environment. After 13 days larvae at 10° C were apparently full grown but had not matured. However, when these larvae were warmed to 25° C they almost immediately went into lethargus and within 6 hours had reached the infective stage. Under natural conditions this would mean that larvae could develop slowly at low temperatures and with the coming of a warm spell development would be greatly accelerated resulting in a rapid transformation of these forms to the infective stage. Table V and Fig. 3 show the relationship between temperature and the number of days required for the larvae to reach the infective stage. The resulting curve appears to be hyperbolic, which suggests an inverse relationship of one of these values to the other. Figure 4 shows the results of plotting the reciprocal of the temperature against the number of days required for the larvae to reach the infective stage. The graph is almost rectilinear. From these results it is reasonable to assume that between the limits of 7.5° C and 27° C the time of development of *D. stenocephala*, from hatching to the infective stage, is inversely proportional to the temperature.

Summary

The effects of temperature on the hatching and development of the free-living larvae of *D. stenocephala* have been studied. Ova hatched, and infective larvae developed at as low a temperature as 7.5°C. The optimum temperature for the free-living stages in these experiments was 20° C. A temperature of 37° C was lethal in 77 hours. Between the limits of 7.5° C and 27° C the time of

development to the infective stage was inversely proportional to the temperature. The ability to grow at comparatively low temperatures has obvious survival value for a nematode having free-living stages and an arctic and subarctic distribution.

Acknowledgments

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NUTRITION OF THE PALE WESTERN CUTWORM, AGROTIS ORTHOGONIA MORR. (LEPIDOPTERA : NOCTUIDAE)

I. EFFECTS OF UNDERFEEDING AND ARTIFICIAL DIETS ON GROWTH AND DEVELOPMENT, AND A COMPARISON OF WHEAT SPROUTS OF THATCHER, TRITICUM AESTIVUM L., AND GOLDEN BALL, T. DURUM DESF., AS FOOD[1]

A. J. McGINNIS[2] AND R. KASTING[3]

Abstract

Larvae of the pale western cutworm fed ad libitum on Thatcher wheat sprouts required six instars to mature and increased in weight from 0.25 mg to near 700 mg in about 4 weeks. Larvae fed for only 2 hours each day required an additional instar, developed more slowly, and were smaller. The growth curves were generally similar as both exhibited a plateau before each molt, followed by a rapid rise. Of four artificial diets tested, only the two containing wheat-leaf juice permitted weight increases. The percentages of dry matter and nitrogen in the cutworm varied according to the stage of larval development and the variety of wheat sprouts fed. These factors, as well as underfeeding, also influenced the efficiency with which the cutworm converted its food to body tissue.

Introduction

The pale western cutworm, *Agrotis orthogonia* Morr., is a pest of the plains area of North America. This insect was chosen for nutritional studies because it can be reared easily in the laboratory (5, 6) and because of its rapid growth and large final size. The present study was undertaken to determine quantitatively the weight changes of the cutworm under controlled conditions. The effects of underfeeding on growth and development were investigated and sprouts of hard red spring (*Triticum aestivum* L. emend Thell.) and durum (*T. durum* Desf.) wheats were compared as food. Results of attempts to rear this insect on artificial diets are also presented.

Materials and Methods

As the cutworms were fed fresh sprouts daily, it was necessary that the sprouts be as uniform as possible to permit quantitative measurements of dry matter and nitrogen intake. Samples of size 8* and size 9* kernels of Thatcher, *T. aestivum* L., and Golden Ball, *T. durum* Desf., respectively, were soaked in distilled water for 2 hours and germinated on moistened filter paper in pyrex dishes. The kernels were sprayed daily with 15 or 20 ml of distilled water. The dishes were enclosed in plastic bags and stored in the dark at 25° C. After 96 hours for Thatcher and 120 hours for Golden Ball the sprouts were separated from the kernels and roots and fed to the cutworms.

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Contribution No. 115, Regional Research Station, Canada Department of Agriculture, Lethbridge, Alberta.
[2,3]Crop Insect Section and Chemistry Section, respectively.
*Kernels retained by sieves with 8/64- and 9/64-inch round holes.

The mean percentages of dry matter and nitrogen in the two varieties of sprouts were as follows:

	Dry matter	Nitrogen (dry weight basis)
Thatcher	9.2	5.44
Golden Ball	11.0	4.60

The differences between the means were both significant at the 1% point.

The larvae were hatched from eggs laid by females collected from the field or by females reared in the laboratory, as described by Jacobson and Blakeley (6). Except where noted, all the insects on test were reared individually in 60×15 mm petri dishes. The larvae were transferred daily to clean dishes and supplied with fresh food.

In the first experiment newly molted fourth-instar larve were used. A group of 20 larvae had fresh Thatcher wheat sprouts available for 2 hours each day whereas the control group of 10 larvae was provided with similar food ad libitum. All insects were weighed daily and their development was recorded until they emerged as adults.

In the second experiment, sprouts of Thatcher and Golden Ball were each fed ad libitum to 50 newly hatched larvae. The insects were reared in groups of about 10 per petri dish, and molting, quantity of food consumed, and body weight were recorded on a group basis until they molted to third instar. Fourteen newly molted larvae from each series were then placed individually in petri dishes and supplied with a weighed quantity of sprouts. After 24

TABLE I

Percentage composition of artificial diets fed to fourth-instar cutworms

	Diet			
	1	2	3	4
Vitamin free casein	—	6.1	6.1	—
Yeast extract	—	0.6	0.6	—
Glucose	—	6.1	6.1	—
Cellulose powder	33.3	26.3	26.3	33.3
Lanolin	—	0.1	0.1	—
Corn oil	—	0.2	0.2	—
Amino acid mixture†	—	1.6	1.6	—
Vitamin solution‡	—	37.0	37.0*	—
Salt solution§	—	22.0	22.0*	—
Plant extract‖	—	—	47.0*	66.7
Water	66.7	—	—	—

*These three solutions were reduced to 55% of original volume at 45° C *in vacuo* to keep the final moisture content at 59%.

†Composed of two parts by weight of each of the following amino acids: DL-alanine, L-arginine, L-glutamic acid, glycine, L-leucine, L-proline, DL-serine, DL-tryptophan, and DL-valine, and one part by weight of each of the following: L-aspartic acid, L-cystine, L-histidine, hydroxy-L-proline, DL-isoleucine, L-lysine, DL-methionine, L-phenylalanine, DL-threonine, and L-tyrosine.

‡Concentration of vitamins in solution (μg/ml): thiamin 5, riboflavin 15, pyridoxine 15, inositol 100, nicotinic acid 15, choline chloride 100, Ca pantothenate 15, folic acid 5, and biotin 0.025.

§Contains 1.8 g NaCl, 0.04 g $CaCl_2$, 0.04 g KCl, 0.04 g $NaHCO_3$, 1.35 g $NaH_2PO_4.H_2O$, and 1.55 g K_2HPO_4 per liter.

‖Juice was pressed from Golden Ball wheat leaves collected when plants were in the milk stage and was kept frozen until required. Upon thawing of the juice, the sediment was centrifuged down and the supernatant fluid was added to the diets.

hours the dry matter content of the sprout remnants in each dish was determined by drying *in vacuo* at 60° C for 5 hours (2). Two samples of fresh sprouts were also analyzed daily for percentage of dry matter. Three samples of sprouts were analyzed daily for nitrogen by the micro-Kjeldahl procedure (2). Insects at different stages of development were also analyzed for dry matter and nitrogen contents by the above methods. Analyses were done on bulked samples of newly hatched and second-instar larvae, individually on three larvae at each of the third, fourth, and fifth molts, and on the survivors 5 days after the fifth molt for each series. The prepupal and pupal forms were from another experiment.

In a third experiment 9 or 10 newly molted fourth-instar larvae were provided with excesses of the artificial diets shown in Table I. The diets were prepared at the beginning of the experiment and stored at −20° C. Moist blotting paper disks were placed in the bottom of the rearing dishes to keep the diets from drying out. The larvae were weighed every second or third day but larval development was recorded daily.

Results and Discussion

In experiment 1 the weight changes that occurred during the life of the cutworm, when the larvae fed on a good natural food under laboratory conditions, were obtained as a guide for further nutritional studies. Newly hatched, newly molted second-instar, and newly molted third-instar larvae averaged about 0.25, 1, and 5 mg. Newly molted fourth instars weighed about 25 mg and achieved a final weight near 700 mg in about 3 weeks when fed ad libitum on Thatcher sprouts (Fig. 1, A). Figure 1,B shows that when food was available for only 2 hours each day the larvae developed more slowly and weight gains and final size were reduced. The premolt plateaus were longer for the underfed insects. Thus standardized feeding periods are necessary when diets are being compared; otherwise it may be difficult to determine whether differences result from the composition of the diet or from the length of the period during which food was available. As a convenience larvae on subsequent tests were fed ad libitum.

The weights plotted in Fig. 1 are means for particular stages of development. For example, the underfed larvae molted for the fourth time 6, 7, or 8 days after the third molt but the weight plotted was the average of the individual weights on the day molting was noted. The weights were similarly averaged for all molts, for maximum weight, and for pupation. In addition, except for the third molt, averages are shown for each of the 2 days before and, except for pupation, for each of the 2 days after a physiological stage. These computed weights were plotted against the average number of days from the third molt to the particular stage. In the figure, arrows indicate the molts, and solid lines connect the values around a particular stage whereas the dashed lines are included for continuity. These composite curves were generally similar in shape to the growth curves for individual insects. The two curves in Fig. 1 show that a plateau occurred before each molt and that weight was gained

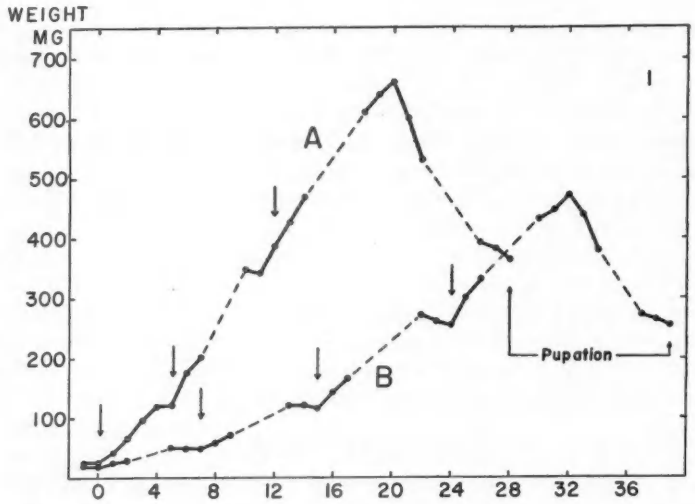

FIG. 1. Mean weights of pale western cutworms reared from the third molt to pupation on fresh Thatcher wheat sprouts. Curve A: Food available ad libitum. Curve B: Food available 2 hours per day.

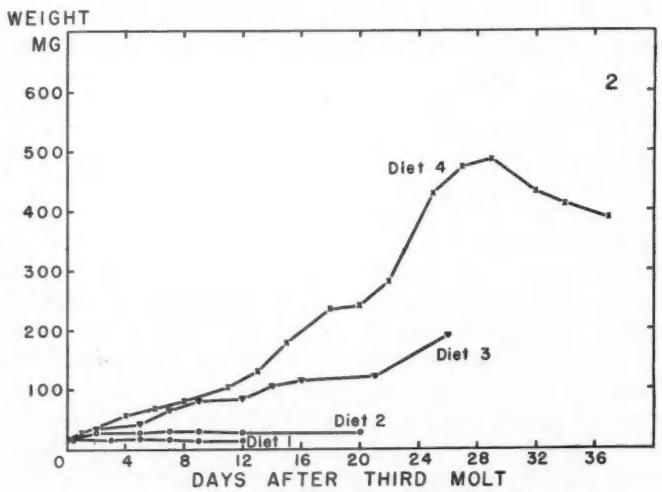

FIG. 2. Weight changes of pale western cutworms fed on artificial diets beginning at the third molt. See Table I for composition of diets. Diet 1, cellulose; diet 2, casein plus; diet 3, casein plus + leaf juice; diet 4, cellulose + leaf juice.

TABLE II

Effect on weight and development of underfeeding Thatcher wheat sprouts to pale western cutworms

No. and sex of insects	No. of instars	Weight (mg)		Period (days)			
		Larval maximum	Pupal	Feeding*	Prepupal	Pupal	Total
Food available 2 hours per day							
5♂	7	463	251	27	7	23	57
11♀	7	468	250	33	7	23	63
2♂	6	333	162	24	8	20	52
1♀	6	331	208	31	5	23	59
Food available ad libitum							
3♂	6	638	360	18	10	25	53
5♀	6	679	365	19	9	25	53

*Third molt to prepupal stage.

rapidly afterward. These detailed changes in weight around the molts were not evident when the data were plotted chronologically.

Table II shows that 16 of the 19 insects on the lower level of nutrition required an extra instar. Despite the differences in maximum larval weight due to the level of nutrition the percentages of weight lost during the prepupal period were comparable and averaged near 45%. The prepupal and pupal periods were generally slightly shorter for the smaller insects and thus tended to compensate for the extra stadium. The level of nutrition during the test period apparently had no effect on the sex ratio. There was little difference in development time between the sexes when the larvae were fed ad libitum, but when food was available for only 2 hours each day, the males completed development more rapidly than did the females. On the average the underfed females consumed more dry matter (251 mg) than did the underfed males (208 mg), yet did not gain appreciably more weight. It is possible that under conditions where food is scarce the female has to feed longer than the male to ensure the necessary reserves for subsequent egg formation.

Initially the larvae were caged in petri dishes with aluminum foil liners. During the 22-hour period when no sprouts were available, pieces of aluminum foil were devoured by some of the larvae and subsequently aluminum pellets were excreted without any apparent effect on development. The use of aluminum liners was discontinued, but this observation indicated that the feeding stimulus was strong and that larger larvae could consume diets that are tough and dry.

Figure 2 shows the growth responses of newly molted fourth-instar cutworms fed on the four artificial diets described in Table I. The terminal point of the curve represents the average survival period of the insect population on a particular diet. The weights recorded are averages of the surviving insects.

All 10 larvae on diet 1 molted on the second day of the test but lost weight throughout and all were dead by the 14th day. Diet 2 was also inadequate for pale western cutworms; only three of the nine cutworms on this diet molted once, five were dead by the 16th day, and one survived for 33 days. Diet 3, which contained wheat-leaf juice, was a much-improved medium. Of the nine insects on this diet all survived for 17 days, seven were dead by the 34th day, two pupated, and one emerged. In addition to the high mortality, several insects initiated a molt but failed to shed the exuvium completely. Diet 4 contained only cellulose powder and wheat-leaf juice, yet permitted the best development. All nine larvae molted at least three times and showed greater weight increases and longer survival periods. Seven larvae died during the prepupal period, two pupated, and one emerged. Whether the high percentage of mortality during the prepupal stage was a direct nutritional effect is not known. These results suggest that the defined diets not only lack nutrients required by the developing cutworm but also contain a factor(s) detrimental to the growing larvae. Although diet 4 was inferior to fresh Thatcher wheat sprouts as a food for the pale western cutworm, it was superior to the other diets tested. This is in direct contrast to results obtained with the wheat stem sawfly (7), where incorporation of wheat-stem juice resulted in less growth and shorter survival periods.

The rapid weight gains made by the pale western cutworm during the larval period were accompanied by changes in body composition as shown in Table III. In general, larvae fed on Golden Ball sprouts contained a higher percentage of dry matter than did those fed on Thatcher sprouts. Conversely the percentage of nitrogen on a dry-weight basis was lower in the series fed on Golden Ball. On a fresh-weight basis, however, the percentages of nitrogen in the two groups of insects were similar. Larvae fed on Thatcher sprouts

TABLE III

Percentages of dry matter and nitrogen in different stages of the pale western cutworm fed on two varieties of wheat sprouts

Stage	Thatcher		Golden Ball	
	Dry matter	Nitrogen*	Dry matter	Nitrogen*
Newly hatched larvae	16.3	9.8	16.3	9.8
1st-molt larvae	13.8	10.0	18.6	8.7
2nd-molt larvae	13.3	—	14.6	—
3rd-molt larvae	14.0	10.1	13.1	9.8
4th-molt larvae	14.5	9.3	16.0	8.0
5th-molt larvae	17.2	8.3	19.7	7.8
5 days after 5th molt	20.3	6.6		
Early prepupal	29.9	6.5		
Early pupae	35.3	7.9		

*Dry-weight basis.

were significantly heavier during the sixth instar than those fed on Golden Ball but dry weights were not different.*

Table III also shows that as the larvae developed there was a general increase in the percentage of dry matter accompanied by a decrease in the percentage of nitrogen. On a fresh-weight basis, however, the nitrogen percentage was relatively constant during the feeding stages. The decrease in weight between the stages of maximum weight and pupation was concomitant with a sharp rise in the percentage of dry matter, indicating that moisture was the major component lost during the prepupal period. There was a lower percentage of nitrogen in the prepupae than in the pupae, an observation that agrees with work done on the Japanese beetle, *Popillia japonica* Newman (1). This relative increase in the nitrogen percentage, during a non-feeding stage, suggested that little or no nitrogen was lost. It is probable that the nitrogen constituted a larger percentage of the total dry matter because carbohydrates and fats were utilized to supply energy for the physiological changes.

TABLE IV

Quantities of food consumed and efficiencies of food utilization by different stages of the pale western cutworm when fed on wheat sprouts

	Mg dry matter consumed*			Mg larval fresh weight gain per mg dry matter consumed		
Instar	Thatcher (2 hr./day)	Thatcher (ad lib.)	Golden Ball (ad lib.)	Thatcher (2 hr./day)	Thatcher (ad lib.)	Golden Ball (ad lib.)
2	—	2.1± 0.6	2.5± 0.6	—	2.5	2.0
3	—	11.3± 4.2	16.6± 6.3	—	2.5	1.8
4	9.2± 2.6	43 ± 7	48 ±10	3.5	1.9	1.6
5	36 ±13	157 ±24	174 ±29	2.1	1.9	1.4
6	81 ±26	282 ±16	287 ±28	1.7	1.0	0.8
7	154 ±28	—	—	1.4	—	—

*Mean consumption and standard deviation.

Table IV shows that when larvae were allowed food for only 2 hours each day they consumed less than those insects fed ad libitum but they made more efficient use of it. When the food was limited it is possible that it passed through the digestive tract more slowly and, therefore, may have permitted better utilization. These data also show that the older larvae, regardless of level of nutrition, were less efficient than the younger ones in converting food to body tissue. The data also show that when larvae were fed ad libitum, Thatcher sprouts permitted greater weight increases per unit of dry matter consumed than did Golden Ball sprouts. The increased efficiency of the insects fed on Thatcher sprouts resulted, in part, from the higher moisture content of these larvae. Even on a dry matter basis, larvae fed on Thatcher sprouts showed a greater gain in weight per unit of food consumed than did

*Unpublished data.

those fed on Golden Ball. These data, along with general biological observations, showed a small but consistent trend favoring Thatcher over Golden Ball sprouts as food for pale western cutworms.

Most of the food was consumed during the last two instars and likewise most of the increase in weight occurred during these stages. Thus data collected over the whole growth period without considering the stage of development would be seriously biased. Although data collected during the early stages were difficult to obtain and were subject to proportionally greater errors, consistent trends have appeared; for example, the decrease in efficiency of utilization of food with age, which would otherwise have been missed. Such trends may be important because it has been shown that newly hatched larvae of some species of insects do not develop on diets that are adequate to permit the development of later instars (3, 4).

Conclusions

1. The effects of underfeeding on growth and development of the pale western cutworm demonstrated that standardized feeding periods are necessary for comparative nutritional studies.

2. Composite growth curves plotted on a physiological basis represent those of individual larvae better than do composite curves plotted chronologically.

3. None of the artificial diets tested was capable of supporting cutworm development comparable to that of fresh wheat sprouts. The results obtained suggest that the diets are chemically inadequate rather than physically unacceptable.

4. The percentages of dry matter and nitrogen in the cutworm varied according to the stage of insect development and the variety of wheat sprouts fed.

5. Stage of larval development, level of nutrition, and variety of wheat sprouts fed affected the efficiency with which this species converted its food to body tissue.

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ROW-TYPE OVIPOSITION IN NEODIPRION SAWFLIES AS EXEMPLIFIED BY THE EUROPEAN PINE SAWFLY, N. SERTIFER (GEOFF.)[1]

A. W. GHENT[2]

Abstract

Many *Neodiprion* sawflies lay their eggs in a row in small pockets cut into the edges of the mature needles of various pines. These rows of eggs are remarkable for the regularity of the spacing between successive egg pockets. The regularity of egg spacing by *N. sertifer* is shown to arise from a stereotyped pattern of leg movements during the shift between the sites of successive egg pockets. Spacing is effected by two sets of leg movements, in each of which the three pairs of legs move forward in order from back to front. While the effective forward movement of all legs is the same, the total forward movement of the metathoracic legs is greater than that of the other legs by an amount equal to the length of an egg pocket. The metathoracic legs retrace their steps by this length as the initial incision for each new egg pocket is cut, evidently providing the force by which this incision is made. The grip-points of the legs are on the needle edge opposite to that receiving eggs, so that the wider the needle, the greater the proportion of leg length required to reach across it, and the smaller the proportion of leg movement reflected in the spacing between eggs. In this way egg spacing varies inversely with needle width. Observations on copulation, antennal movement, and related oviposition behavior are included.

Introduction

The *Neodiprion* sawflies deposit their eggs in the edges of the needles of various conifers, in small pockets cut by the saw-like lancet of the adult females. This basic operation of cutting an egg pocket and depositing a single egg therein appears to be constant throughout the genus, but the location and grouping of these egg pockets is variously modified. Current knowledge of these modifications permits the definition of at least three distinct types of *Neodiprion* oviposition behavior.

Type 1: *A single egg pocket at or against the basal sheath of the needles of various pines.* This behavior pattern appears to be restricted to the members of a phylogenetic unit set up by Ross (11). It has now been confirmed that three of the four species grouped by Ross, i.e. *N. swainei* Midd. (5), *N. hetricki* Ross (5, 6), and *N. excitans* Roh. (personal communication, L. A. Hetrick, 1958), share this behavior. Scanty records (9) suggest that the fourth species, *N. pini-rigidae* (Norton), behaves in a similar way.

Type 2: *A single egg pocket at or about the mid-point of the mature needles of various conifers.* At present only two species are definitely known to display this behavior: *N. abietis* complex, which lays its eggs on various firs and spruces; and *N. tsugae* Midd. (7), which lays its eggs on western hemlock, *Tsuga heterophylla* (Raf.) Sarg.

[1]Manuscript received February 24, 1959.
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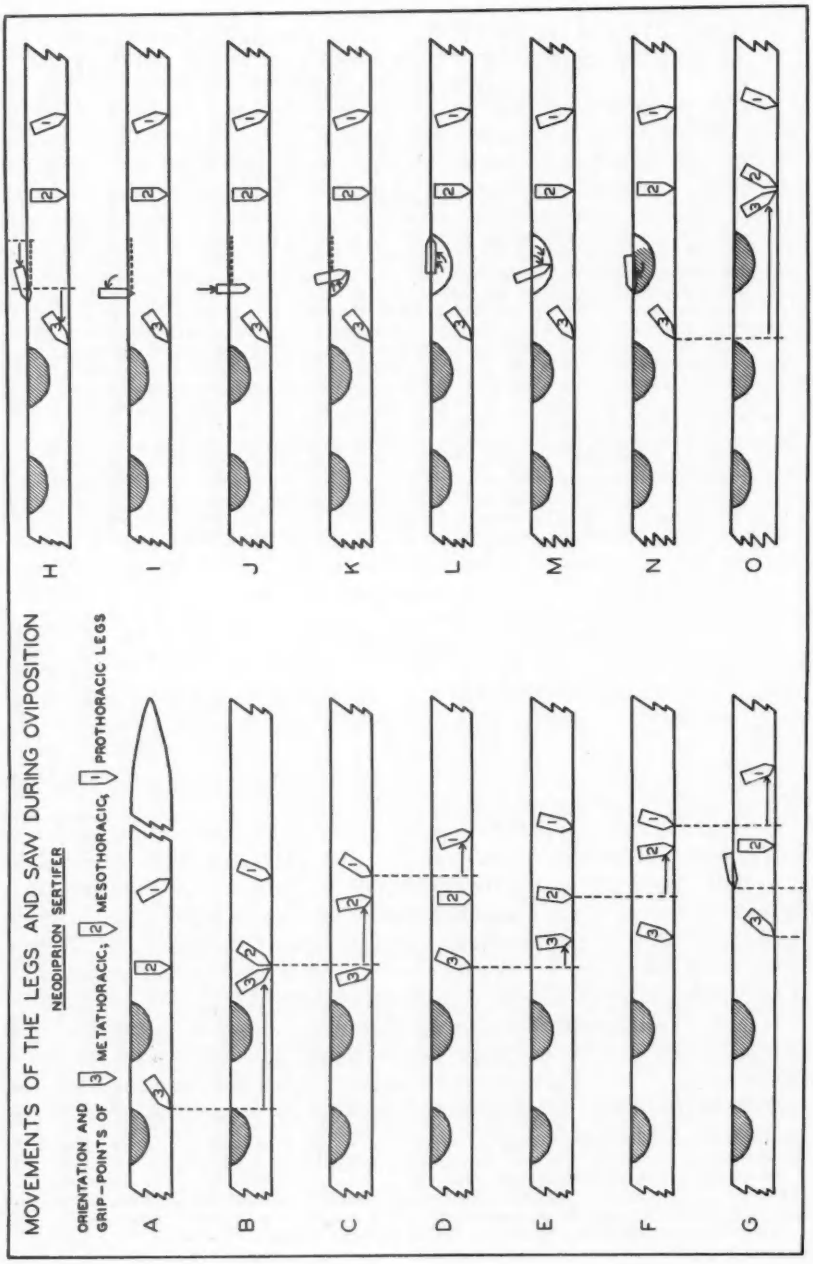
MOVEMENTS OF THE LEGS AND SAW DURING OVIPOSITION
NEODIPRION SERTIFER
ORIENTATION AND GRIP-POINTS OF 3 METATHORACIC; 2 MESOTHORACIC; 1 PROTHORACIC LEGS
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O

FIG. 1.

Type 3: *A row of regularly spaced egg pockets on the mature needles of various pines.* The present paper is concerned with this row-type oviposition behavior. Most of the pine sawflies for which any record of the oviposition pattern is available display this behavior, including such well-known forest defoliators as *N. lecontei* (Fitch), *N. pratti* sspp., *N. n. nanulus* Schedl, *N. virginianus* complex, and *N. sertifer* (Geoff.). The appearance of the row of egg pockets cut by these species can be appreciated from Fig. 1 in this paper, or more adequately from the illustrations in Atwood and Peck (1).

The spaces between successive egg pockets in this row-type oviposition, while not *exactly* constant, are yet so strikingly regular as to have drawn the attention of every writer who has described these species. The behavior that gives rise to this regular spacing, however, has remained undescribed. The Forest Insect Laboratory in Sault Ste. Marie has recently undertaken studies of the European pine sawfly, *N. sertifer*, at a small field station some 15 miles south of Owen Sound, in Southern Ontario. In this area *N. sertifer* has become a serious threat to reforestation projects and to private plantations of Scots pine, *Pinus sylvestris* L., largely intended for early harvest as Christmas trees. Studies continued into midautumn of 1958 in this area provided the opportunity to observe the row-type oviposition behavior of this sawfly. Study of this behavior disclosed that the regularity of egg spacing arises from a stereotyped pattern of leg movements associated with the cutting of the egg pockets. In the hierarchy of alternatively descriptive and explanatory levels in biology, we must look to the description of these leg movements for our immediate explanation of the regular spacing of the eggs. The mechanisms governing the regularity of the leg movements themselves, though open now to some potentially profitable speculation, remain formally untouched by the present study.

FIG. 1.

Movements of the Legs and Saw during Oviposition

Figure 1 illustrates the movements of the legs of *N. sertifer* females while shifting between the sites of successive egg pockets, and the movements of the saw during the cutting of an egg pocket. The cutting of an entire row of egg pockets is a simple repetition of these movements. Females remain oriented towards the needle tip without interruption while ovipositing. Their legs grip the needle edge opposite to that receiving eggs, and usually, though not invariably, they hang below the needle. In this way the grip-points of the legs are usually on the gravitationally upper needle edge, while the egg pockets are cut on the lower edge. This is discussed in greater detail in a later section. Figure 1 is drawn as if the eggs were being laid on the upper edge of a needle, and though this is infrequently the case, the diagrams are more readily followed in this position by anyone not already familiar with their content.

At rest the grip-points of the legs of an adult female are approximately equidistant: the metathoracic legs grip just below the end of the abdomen, the mesothoracic legs directly below their point of attachment, and the prothoracic legs at a point below the compound eyes. In the following

descriptions of leg movement it is convenient to refer to the legs as adopting momentarily the "resting" position, but this should not be taken as implying any cessation of activity. Movements of the legs between egg pockets, and movements of the saw while cutting an egg pocket, are divided into 15 stages lettered from "A" to "O" in Fig. 1.

Stage A.—As an arbitrary starting point in a continuous pattern of movement, diagram A illustrates the grip-points of the legs at a time when the egg pocket (shown between the metathoracic and mesothoracic legs) has been cut, and the egg itself has been deposited. The abdomen of the female has straightened from the flexed position maintained while cutting the egg pocket and depositing the egg, and is not, at this point, in contact with the needle edge. This stage lasts only a few seconds, as the first leg movements begin immediately after the abdomen is straightened. The grip-point of the metathoracic legs is shown to be directly opposite the apical end of the *previous* egg pocket. Though minor variations occur, this is commonly the case. In achieving this grip-point the metathoracic legs are fully extended backwards, so that the space between the grip-points of the metathoracic and mesothoracic legs is substantially greater than the space between prothoracic and mesothoracic grip-points. This extension of the metathoracic legs becomes comprehensible in stage H.

Stage B.—The pattern of leg movement in forward motion is a hitching forwards first of the metathoracic legs, then the mesothoracic legs, and finally the prothoracic legs, always in that order. In stage B the metathoracic legs begin the first of two sets of leg movements that determine the separation of the egg pockets along the needle edge. From the fully-extended position described in stage A, the metathoracic legs are brought forward until they touch, or almost touch, the grip-point of the mesothoracic legs. Compared with the leg movements in normal walking, and with the remainder of the leg movements during egg spacing, this first movement of the metathoracic legs is abnormally long.

Stage C.—The mesothoracic legs are now brought forwards, though their displacement is only about half that of the metathoracic legs in stage B.

Stage D.—The prothoracic legs are now brought forwards, so that the grip-points of the three legs become approximately equidistant. The legs are "bunched" however, the grip-points being closer together than they normally are when the insect is at rest. This bunching appears to result from the abnormally long forward movement of the metathoracic legs in stage B. The next set of three leg movements, shown in the next three diagrams, appears to be in compensation for this bunching. Very infrequently this next set of "compensatory" movements does not occur, and oviposition proceeds immediately from this point.

Stage E.—Diagram E illustrates the first of these "compensatory" leg movements. Again the metathoracic legs are the first to be brought forward, though this time only a part of the way towards the mesothoracic legs.

Stage F.—The mesothoracic legs are again moved part way toward the prothoracic legs.

Stage G.—The prothoracic legs are brought forwards to balance the spacing of the metathoracic and mesothoracic legs, so that the grip-points are again approximately equidistant, and spaced more nearly as they are in the resting position. Very rarely this second set of leg movements will be followed by a third of the same order, but these have not been illustrated. At this point the abdomen is flexed and the tip of the saw, which is oriented caudally when at rest, is caught against the needle surface.

Stage H.—The explanation of the extreme extension of the metathoracic legs during oviposition becomes apparent in stage H. The initial incision is made in the needle tissue from what will be the apical end of the egg pocket back towards its basal end. The tip of the saw-like lancet, shown as it was caught against the needle tissue in stage G, is now pulled backwards during stage H as the metathoracic legs keep gripping and regripping the needle. The force by which the initial incision is cut appears to be supplied through this pulling action of the metathoracic legs as they are extended backwards, so that the length of the egg pocket is governed by the difference between the resting and the fully extended grip-points of the metathoracic legs.

Stage I.—Following the initial incision, the female straightens her abdomen. In this way the saw, still caught by its tip in the needle tissue, comes to stand at right angles to the needle edge.

Stage J.—From this position the saw is worked straight in, until one-half to two-thirds of its length is within the needle.

Stage K.—The first motion of the saw is forwards, towards the apical end of the incision. Seen by transmitted light, a band of hollowed egg pocket begins to appear along the entire trailing edge of the saw. As the saw advances, this band broadens at the saw tip, so that it becomes apparent that the saw is describing an arc.

Stage L.—The forward and upward swing of the saw brings it to a position in which the saw tip is oriented apically and parallel to the needle edge. Nearly half of this 180° arc described by the saw is accomplished through the flexure of the end of the abdomen, which at this point is bent almost at right angles to the forepart of the body.

Stage M.—The saw is returned towards the basal end of the egg pocket in a series of short, quick, wiping motions against the inner walls of the pocket.

Stage N.—As the saw returns to its original position, directed caudally and again parallel to the needle edge, the female squats tightly against the needle, and by transmitted light the egg may be seen to be thrust into the egg pocket from the basal end and to slide forward until it comes to rest against the apical end of the pocket. Middleton (8) suggested that the valves of the saw might be used to hold open the edges of the pocket while the egg is "dropped" into place. The females squat so tightly against the needle that there seems to be no obvious way in which this suggestion can be confirmed.

Yet since the needle epidermis is only cut, without any appreciable part of its substance being torn away, and closes over the egg pocket after the egg is laid, Middleton's suggestion is probably sound. Apart from the egg, shown only partly filling the egg pocket, and the saw, shown still in contact with the edge of the egg pocket as Middleton envisages, stage N as pictured is identical with our starting point, stage A.

Stage O.—With the addition of a third egg pocket, diagram O is identical with diagram B, and is included solely to link up the continuity of events with our arbitrary starting point. The European pine sawfly usually continues this process until seven or eight eggs are laid. Occasionally on long needles, or when the first egg has been laid very near the base of the needle, as many as 11 or 12 eggs may be laid in the row.

The length of the egg pocket itself has been described as being equal to the difference between the position of the resting and fully extended grip-points of the metathoracic legs. The space between eggs is derived in a more complicated way, and it is again pertinent to describe this in relation to the movements of the metathoracic legs. The *effective* forward movement of all legs is of course the same, and is equal to the combined length of a space and an egg pocket. The *total* forward movement of the metathoracic legs, however, is equal to the combined length of a space and two egg pockets, since the metathoracic legs retrace their steps by an amount equal to the length of an egg pocket as the initial incision is cut. The extra distance traveled by the metathoracic legs is incorporated in their first abnormally long forward movement following oviposition. The length of the space between egg pockets may therefore be defined as the total forward movement of the metathoracic legs less the length of two egg pockets.

Variation in Egg Spacing with Needle Width

An unusual egg cluster attracted atention early in the 1958 field season, before the foregoing observations had been made. This cluster had been laid (in the autumn of 1957) on the terminal of a small shade-grown Scots pine, underplanted in a grove of white cedar (*Thuja occidentalis* L.). The needles on this pine were long and spindly, and appreciably narrower across their flat surface than is customary even for the needles of red pine, *P. resinosa* Ait. The egg pockets on these needles were strikingly farther apart than any that had been observed in two- to three-hundred egg clusters examined more or less closely in the course of the previous month's work. The wide spaces between eggs on these narrow needles were recalled during the study of leg movements during oviposition, when their probable explanation became apparent. Oviposition proceeds while the legs are fully extended across the needle width. It should follow, then, that the less that width may be the smaller the proportion of leg length required to reach across the needle, and the greater the proportion of leg length available for movement forwards and consequently for egg spacing. It appeared, in other words, that egg spacing should vary inversely with needle width.

A study of 68 egg clusters from Scots, red, and jack (*P. banksiana* Lamb.) pines confirmed that in essence this is true. The regression and correlation between needle width and egg spacing is illustrated in Fig. 2. For ease in measurement, egg spacing is given as the length of needle per egg, and includes throughout a factor of approximately 2 mm for the length of the egg pocket. The plotted points represent cluster averages. For each cluster the 10 to 12 needles bearing the greatest number of eggs were cut crossways with a razor blade, at the apical ends of the most basal and the most apical eggs. In this way a needle bearing, for example, seven eggs, yielded a single measurement of the combined length of six eggs and six spaces. These measurements were totalled and averaged over the 10 or 12 needles representing the cluster. In addition the width of each egg-bearing needle was measured at its mid-point, and again averaged over the group. The needles from any one cluster, being on the same shoot of foliage, display virtually no variation in width, so that the accuracy of the means plotted along the abscissal scale is unusually high. Similarly the egg-spacing over the cluster is remarkably constant, so

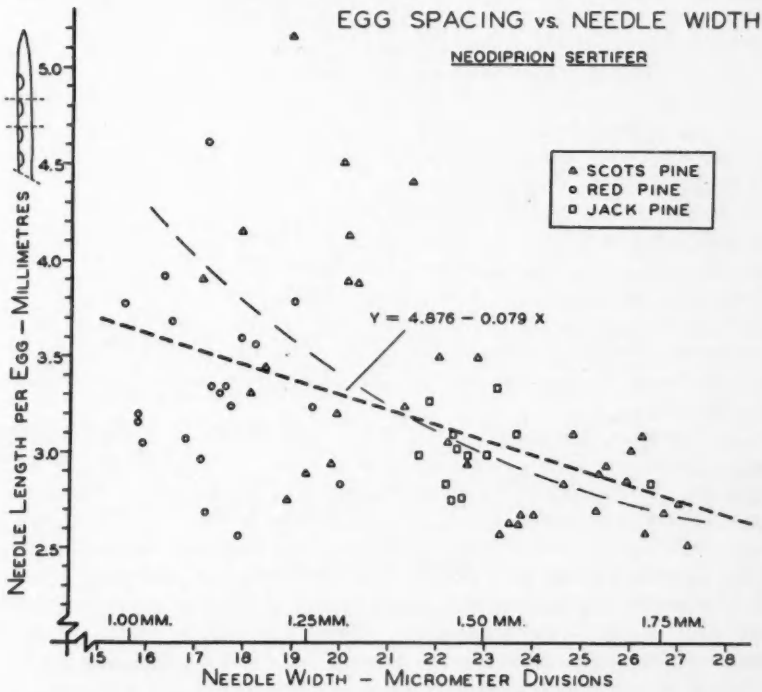

FIG. 2. Correlation and regression, egg spacing vs. needle width. $r_{x \cdot y} = -0.478 \pm 0.094$, significant beyond the .001 level. The arc of the circle, the theoretical locus of the relation, is fitted by eye and serves only to illustrate the orientation of the arc with respect to the two axes.

that the mean obscures no very large variance that might render a correlation analysis meaningless, and is the best figure to represent the cluster.

In Fig. 2 the extreme variance about regression on the narrower needles holds the correlation coefficient to a relatively low value, -0.478 ± 0.094. With 65 degrees of freedom this correlation is significant beyond the .001 level. Squaring the coefficient suggests that we may account for approximately 25% of the variance in egg spacing through correlation with needle width, the remainder being ascribable to differences in the size and behavior of the adult females.

It is apparent from Fig. 2 that variance about regression is greatest for the narrowest needles, and that variance decreases steadily as needle width increases. I suspect that this reflects a certain selection made in effect by the foliage for females that are capable of laying on it. Considerable variation exists in the size of adult females. While all females are evidently capable of laying on narrow foliage, so that both wide spacing (larger females) and close spacing (smaller females) is observed, it is likely that only large females are capable of laying on wide needles. Very wide needles force a pattern of the closest possible spacing even upon what may be presumed to be the largest females. Several clusters that averaged 27 micrometer divisions (approximately 1.75 mm) in needle width are represented in Fig. 2. Several individual needles from these clusters were as wide as 29 micrometer divisions, and many of the egg pockets in these needles ran into each other, the female having failed to effect sufficient spacing to keep the apical and basal ends of successive egg pockets apart. Many Scots pine in southern Ontario bore extremely wide needles on the 1958 shoot growth, some entire shoots being characterized by needles of 2 to 2.5 mm in width (roughly 32 to 40 micrometer divisions on the scale of Fig. 2). Special efforts were made to find egg clusters on these wide-needled shoots in areas of heavy oviposition on the more normal foliage, but none could be found. These very wide needles are evidently immune to oviposition by the European pine sawfly, a conclusion that holds out some promise as an avenue of selection for sawfly resistance in tree-breeding programmes.

The regression illustrated in Fig. 2 has been shown as a simple straight line, a description that is adequate over the narrow range of needle widths available. Theoretically, however, the locus of the relation is the arc of a circle, oriented concave-side upwards as sketched in Fig. 2. It becomes apparent that the relation is circular if one thinks of the insect as being anchored at the distal end of the fully extended metathoracic legs, and then visualizes the needle widening beneath the insect, so that the entire body of the insect must shift upwards and backwards. The length of the metathoracic leg, or at least some constant fraction thereof, becomes the radius of a theoretical quarter circle with imaginary limits at right angles to each other. One may imagine these limits being imposed by a needle of negligible width on the one hand, and on the other by a needle so wide that the leg might reach across it only by being extended straight down from its point of attachment.

Gripping of the Needle during Oviposition

The grip-points illustrated in Fig. 1 are shown from one side of the needle only, and essentially this one-sided picture is sufficient, as the grip-points of left and right legs align within a small fraction of a millimeter of each other. Yet in the order of gripping during the cutting of an egg pocket there is an interesting balance, for if all three left legs were to grip even slightly ahead of all three right legs, for example, a torque would arise that would pull the insect to one side. Trivial as this error might seem to be, it is still one that appears never to be committed. Invariably in these observations the gripping-order of one of the three pairs of legs was the reverse of that of the other two. This statement is based on a run of over 30 successive observations without contrary incident, and achieves a level of significance beyond question. Commonly, though not invariably, the mesothoracic tarsi were actually overlapped at a common grip-point, while the grip-order of the prothoracic legs, let us say right before left, was the reverse of the metathoracic legs, left before right. In this way the problem of torque is eliminated.

The needles of two-needled pines, such as Scots, red, and jack, are characterized by having one flat surface that opposes the corresponding flat surface of the second needle in the pair, and one curved surface approximating a semicircle in cross-section. *N. sertifer* makes its incisions in the flat surface of the needle, adjacent to either edge where flat and curved surfaces meet. Visualizing a needle from its flat surface, apex upwards, it is apparent that an insect cutting its egg pockets into the flat surface against the right-hand edge must extend its left legs only the relatively short distance across the flat surface, while the right legs must reach the greater distance around the curved surface. In this circumstance the full value of the tarsal claws becomes apparent, for on wider needles the insect is often unable to reach around the curved surface to the opposite edge, and with one or more of its legs must secure what purchase it can on the curved surface of the needle. For this reason the contention that the larger needles are, in effect, "selecting" for the larger females that can lay on them is probably valid. Adult size appeared also to influence the rate of egg laying very markedly. On several occasions during the observations of leg movement as many as three or four females were ovipositing on the same shoot in a study cage, and larger females took noticeably less time to cut their egg pockets than the smaller females required.

Copulatory Behavior

Handling of substantial numbers of adult males and females during these studies provided many opportunities to observe them in copula. Middleton (8) noted that female *N. lecontei* "appear to resist the attempts to mate offered by the male", and this appeared to be equally true of *N. sertifer*. Females of this species are shy of making contact with other moving insects whether they be males or females, their reaction to males being more violent as the males, unlike other females, attempt to stay in contact with them.

Effective contact is achieved by the male jumping on to the dorsal surface of the female either from behind or from the side, and advancing forwards until their bodies are congruent, head over head and abdomen over abdomen. At this point the male turns around on the back of the female to face the other way, and literally runs off the end of her abdomen, grasping it at the last moment with his metathoracic legs and opposing the end of his abdomen to hers. Females generally remain motionless for several minutes after the initial contact, but usually renew their walking thereafter. Being larger and more powerful than the males, they may carry the male behind them wherever they go for as long as 10 minutes. Contact is usually broken by the female reaching back with her metathoracic legs and pushing the male away from her. Rejected males often attempt to regain contact immediately, sometimes successfully.

Middleton (8) recorded a similar method by which, on one occasion, a male *N. lecontei* adult arrived in position for copulation. In this instance the male crawled over the female "from head to posterior end". Both Middleton (8) and Rohwer (10) noted that *N. lecontei* adults mate while facing in opposite directions. Hopping and Leach (7) reported that *N. tsugae* adults also face in opposite directions while mating, although their statement that "in copulation the male backs up to the female" should be confirmed by fresh observation. Rohwer (10) recorded the mating habits of four other sawflies in other genera, and of these all but one, a wood wasp, adopted this same orientation for copulation.

Cleaning Behavior

An interesting feature of oviposition by *N. sertifer* is the deliberate brushing away of the ball of "sawdust" that accumulates on the underside of the female during the cutting of a row of egg pockets. This ball of chopped needle tissue may partly crumble and fall away, without help from the female. It remains untouched in any deliberate way, however, so long as the female continues to cut the row of egg pockets. Cleaning follows after the row is finished, and takes the form of a purposive shoving at the ball of sawdust with the metathoracic legs. Large balls of sawdust are always cleaned away in this manner, and even the small fragments that remain when the sawdust ball has crumbled from its own weight are often removed.

The most notable feature of the cleaning activity is its orientation: females observed in this study *invariably faced the needle base* while cleaning. This statement is based on a run of 16 successive observations without contrary incident, a sequence that might occur by chance with a probability of slightly less than 1 in 65,000. The cleaning behavior is evidently completely blocked so long as the female is facing the needle tip, in which position she may either begin or continue to oviposit. It may be essential to the whole oviposition pattern that cleaning should be blocked while the females are so oriented. The laying of a row of eggs is a behavior *unit*, in which a cycle of operations

must be repeated at successive egg pockets without interruption. Disturbances that do occur, such as the attempts of a male to mate with an ovipositing female, bring the particular row of eggs to an end. The female never returns to a half-finished egg pocket once the lancet is removed, but begins a new cycle of egg-laying operations on another needle. It may be argued, then, that if the cleaning behavior could proceed while the females were oriented towards the needle tips, this activity would itself interrupt oviposition, and "rows" of more than one or two egg pockets would never be achieved.

The evidence is conclusive that the oviposition and cleaning activities of *N. sertifer* are dissociated. During the laying of a row of eggs the insect behaves as if it were temporarily "locked in gear" to repeat the cycle of leg and saw movements. Not until the insect becomes "unlocked", at the end of the row of eggs, are other activities possible. Two conditions would permit the inference that cleaning was "locked" into the same behavior unit: it should follow immediately, and it should follow invariably, after oviposition. Neither of these conditions hold. It is not immediate: cleaning may proceed as soon as the insect turns to face the needle base, or it may wait until the same orientation is adopted some minutes later when the insect has wandered to another needle. It is not invariable: as already noted in the opening paragraph of this section, the small fragments that remain when the sawdust ball has crumbled from its own weight are often, *but not invariably*, removed. Cleaning is in some way "triggered" (orientation permitting) by the ball of sawdust, rather than by the cessation of oviposition.

In 1952 I had the opportunity to watch two adult females of the balsam fir sawfly, *N. abietis*, lay a total of 20 eggs. It was my impression at that time that the females began probing at the balsam needles with the lancet whenever they faced the needle tips, and ceased to do so whenever their wanderings over the foliage brought them to face temporarily toward the needle bases. This observation has hitherto gone unreported, but it clearly belongs with the present observations on cleaning and ovipositing by *N. sertifer*. There is evidence in these observations that elements of behavior that are compatible, or elements that are necessarily associated, are brought together under common orientation categories. In this way the incompatible elements of behavior, which must necessarily be dissociated, can be placed in mutually exclusive orientation categories, effectively preventing their interference with each other.

It is reasonable to suppose that sawfly adult behavior might be organized at a more complex level than the behavior of sawfly larvae. In a recent paper (4) on the feeding orientation of larvae of *N. pratti banksianae* Roh., it was shown that under some conditions the photopositive response would take precedence over the response to the free end of the needle, while under other conditions the reverse was true. The independence of the various responses associated with feeding orientation was illustrated by their liability to experimental rearrangement in bizarre combinations. The evidence here suggests

that, for the larvae, there is a hierarchy of independent behaviorisms dictated by the conditions of the moment, rather than a grouping of compatible and necessarily associated behaviorisms. It is not suggested that these patterns of organization are the whole story behind the behavior of either larvae or adults, but they are reflections of the *kind* of organizational structure that pertains to these different levels of development. These simple responses of sawflies may better serve to illustrate the structural organization of instinct than the more widely studied and more complex responses of bees and ants.

Antennal Behavior

The antennae of *N. sertifer* females are constantly active in stroking the sides of pine needles as they wander over the foliage and as they move between the egg pockets of a row. They appear to test for two main features of the needle: the presence of eggs already laid, and the narrowing of the needle at its tip. The first of these is well illustrated by a chance observation. A female was seen to "select" for oviposition a needle that had already received a short row of five eggs in the upper third of its length. The needle was "selected" at a point close to its base, so that the female did not come into prior contact with these previously cut egg pockets. The female backed down the needle, oriented over its flat surface, and in so doing her back was pressed against the opposed flat surface of the other needle of the pair. Her efforts in pushing down between the needles visibly increased the angle between them. She began to oviposit close to the needle base, and continued until a row of six eggs extended apically. It was apparent while she was cutting the sixth egg pocket that her own length was sufficient to bring her head and antennae over the first egg of the earlier series as she moved forward to cut what would have been the seventh egg pocket. The instant her antennae contacted the first egg pocket of the earlier series she stopped, stroked the needle with her antennae along the sides of this earlier pocket for some 4 or 5 seconds, and then turned and abandoned the needle. In this we see the sharpest possible distinction between reasoned and instinctive behavior, for 8 mm of unused needle was found to remain between the sixth egg pocket of the new series and the first egg pocket of the old. In this distance, at least one and possibly two additional egg pockets could have been accommodated, but to have cut these would have required that she stand over egg pockets already cut.

Needles may be "selected" for oviposition at any point along their length, and it is immediately apparent when the female has "decided" that the needle is suitable, for she begins at once to back down the needle, probing constantly with the lancet. Retrograde movement of this sort appears to be uniquely associated with the "decision" to oviposit. G. W. Green (personal communication) observed this same retrograde movement just before oviposition by *N. lecontei* on red pine.

The response of the female, through antennal contact, to the narrowing of the needle at its tip may be observed whenever the regular pattern of leg

movements following the cutting of an egg pocket carries the antennae of the female beyond the point at which the needle begins to narrow. If she reaches this point after the second or "compensatory" set of leg movements, there follows immediately a third identical set of leg movements, and then a fourth, and perhaps even a fifth, the antennae coming progressively closer together until they actually touch each other just beyond the needle tip. At this point the female immediately turns and abandons the needle.

Response to Gravity

In an earlier analysis (3) of the oviposition behavior of *N. p. banksianae*, it was reported that this species displayed a marked tendency to select the lower gravitational edges of jack pine needles for oviposition. Of the 745 needles examined in that study, 86% bore eggs on the lower edge.

An examination of *N. sertifer* egg clusters collected in the autumn of 1958 revealed that this species shares this same tendency. The same protractor fitted with a pendulum needle, described in the 1955 paper (3), was used to measure the orientation of the shoots in the field. Egg clusters were collected from Scots, red, and jack pines and pinned at the appropriate angle to a board in the laboratory for examination in their original orientation. Eight clusters from Scots pine had a total of 147 egg-bearing needles, of which 112, or 76%, bore eggs on the lower edge. Ten clusters from red pine had a total of 182 egg-bearing needles, of which 126, or 69%, bore eggs on the lower edge. Similarly five clusters from jack pine had a total of 104 egg-bearing needles, of which 87, or 84%, bore eggs on the lower edge. Taking the three host species together, 75% of the egg-bearing needles bore their eggs on the lower edge. Approximately 6% of the total of 433 needles from the three hosts had to be classified as "intermediate", so that only 19% of the needles bore their eggs on what was clearly the upper edge.

In the earlier paper (3) it was suggested that this strong trend to lower-edge laying might arise from the greater stability offered the ovipositing female by having its weight slung below the needle. Observations of ovipositing *N. sertifer* females were in no way contrary to this hypothesis.

Discussion

Atwood and Peck (1) stressed the value of between-species differences in egg spacing as diagnostic field characters. On purely empirical grounds they probably would not have claimed that *N. sertifer* egg clusters could be identified on this basis, had this species been included in their study. It is apparent in Fig. 2 (after subtracting 2 mm for the length of the egg pocket itself) that *N. sertifer* egg spacing ranges from those that are separated by nearly one and one-half times their own length to those that are separated by approximately one-quarter of their length only. Scots pine is unusually variable in needle width, markedly more so than either red or jack pine, as

illustrated in Fig. 2. For species restricted to hosts that vary only slightly in needle width, between-species differences in egg spacing are probably valid field recognition characters.

In the section on the gripping of the needle during oviposition, it was noted that the egg pocket of *N. sertifer* is cut into the flat surface of the needle, just in from the needle edge. This position was noted by Brygider (2), who also pointed out that *N. n. nanulus* cuts its egg pocket in the same position. *N. n. nanulus* is shown by Ross (11) to be the most nearly related of the North American *Neodiprions* to the European *N. sertifer*, and one may predict that *N. n. nanulus* will be found to share other behaviorisms with *N. sertifer*, such as the tendency to back down between opposed needles of the pair, forcing them a little farther apart. In contrast, *N. swainei* cuts its egg pocket from the *curved* surface of the needle, again just in from the needle edge, as illustrated by Ghent and Wallace (5). It is likely that the three species grouped with *swainei* by Ross (11), and listed in the introduction, will also be found to cut their egg pockets into the curved surface of the needle. The behavior apparently common to these species is the laying of a single egg close to the basal sheath where the two or three needles in the fascicle are held tightly together, and where presumably only the curved outer surfaces of the needles are accessible.

Brygider (2) gives information on the position of the egg pocket of another *Neodiprion* sawfly, now known as *pratti banksianae*. This sawfly also cuts into the curved surface, just in from the needle edge, in the same manner as *N. swainei*. This characteristic is of the greatest possible value in distinguishing egg clusters of *pratti banksianae* from those of *sertifer* in areas where both occur, as they do in parts of the Lake Huron forest area of southern Ontario. Exhaustive tabulation of the position of *Neodiprion* egg pockets with reference to flat and curved needle surfaces, together with information on spacing, host specificity, geographical distribution, and phenology, may yet make it possible to achieve virtually complete reliability in the identification of egg clusters. It is already known from inspection of Forest Insect Survey collections in Ontario that *N. lecontei* and *N. virginianus* cut their egg slits from the flat surface, in the manner of *N. sertifer* and *N. n. nanulus*. Since *swainei* and *pratti banksianae* egg slits differ in position from those of *virginianus* and *lecontei*, it is possible already to conclude that the position of the egg slit is not consistent within the two major divisions of the *Neodiprions* set up by Ross (11). One might expect it to be consistent within smaller groupings, however, as among the three subspecies of *pratti* presently defined.

The mechanics of leg movement remains for the present a matter of speculation, yet it is possible that it may be of the simplest sort. The first long forward movement of the metathoracic legs may be a response to muscle fatigue, and the second movement, not always undertaken, a compensation for the bunching of the legs following this inordinate initial movement. The "spacing mechanism", under this hypothesis, ceases to exist as such: the forward motion becomes a by-product of muscular tensions built up during

the cutting of the previous egg pocket, and it may be possible to duplicate some of these movements by experimental extension of the metathoracic legs for periods comparable to those involved in oviposition. The row-type oviposition behavior appears to call for only one prerequisite: the ability to lay one egg after another without interruption. Concomitantly, a mechanism capable of blocking this ability for even a few moments may have been the prerequisite to the development of the *swainei–hetricki* and *abietis–tsugae* behavior patterns.

The present study should permit the row-type oviposition behavior of *N. sertifer* to be compared with the behavior of sawflies in the *lecontei* group. Should the movements of the legs and saw prove to be the same in the *lecontei* and *sertifer* groups, we may argue with justification that row-type egg laying is the ancestral pattern. The *Neodiprion* sawflies are an excellent example of a group in which studies of the patterns of both morphological and behavioral kinship may contribute jointly to an understanding of their phylogenetic relations.

Acknowledgments

I wish to thank both H. H. Ross and L. A. Hetrick for correspondence encouraging the pursuit of these studies. The manuscript has profited from critical reading by several members of the Forest Biology Division, notably R. M. Belyea, and frequent discussions with D. R. Wallace and K. J. Griffiths have stimulated much of the work.

I have the happy opportunity to acknowledge the assistance of my mother, Mildred C. Ghent. Her ability to take notes in shorthand permitted me to dictate several thousands of words of descriptive material on the rapid movements of the ovipositing females while my attention remained fixed on the study cages.

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CALIPERIA BREVIPES N. SP. (CILIATA: PERITRICHA), EPIZOIC ON RAJA ERINACEA MITCHILL AT SAINT ANDREWS, NEW BRUNSWICK[1]

MARSHALL LAIRD[2]

Abstract

Two adults of the common skate, or tobacco box, *Raja erinacea* Mitchill, trawled in Passamaquoddy Bay, N. B., in June, 1958, bore myriads of peritrichous ciliates on and about the free edges of the gill filaments. The morphology of these epibionts refers them to the genus *Caliperia* Laird (Scyphidiidae), previously known from a single New Zealand species. *C. brevipes* differs from the genotype in having relatively shorter caudal processes, a posteriorly located micronucleus, and a prominent siderophilous collar at the cytostome. Comparisons are drawn between these organisms and *Ellobiophrya donacis* Chatton and Lwoff.

Introduction

Caliperia longipes Laird was described from the gills of two fishes of the intertidal zone collected near Wellington, New Zealand—a clingfish, *Oliverichtus melobesia* (Phillipps), and a blenny, *Ericentrus rubrus* (Hutton) (6). It is a peritrichous ciliate, characterized by an attachment organelle demarcated from the endoplasm of the zooid and having two caliper-like posterior processes. These contain a rod-shaped structure which runs the length of one of them, curves through 180° just short of the posterior limit of the body proper, and proceeds to the distal extremity of the other. They pass one on either side of a gill filament, grasping it firmly between them, thanks apparently to the elasticity of the recurved rod. Their free ends meet beyond the filament, and are pressed, but not locked, together.

There is close but superficial similarity between *C. longipes* and *Ellobiophrya donacis* Chatton and Lwoff, another peritrichous epibiont from the intertidal zone. *E. donacis* resembles the New Zealand ciliate in securing itself to the host (a European lamellibranch, *Donax vittatus* da Costa) by means of two posterior processes. These, however, contain no supporting organelle, their endoplasm is continuous with that of the zooid, and they interlock distally by means of a button-like structure (1, 2).

Until now, both genera have remained monospecific. Chatton and Lwoff (1) at first regarded their epibiont as the type of a new family, but subsequently (2) referred it to a new subfamily of the Vorticellidae, the Ellobiophryinae, allied to the Scyphidinae. Kahl (4) revised the order Peritricha, raising the Scyphidiidae to the same rank as the Vorticellidae (Peritricha: Sessilia) and transferring *Ellobiophrya* to the former family. Others (5, 6) have followed his lead, and *Caliperia*, too, is placed in the Scyphidiidae (6). It has been

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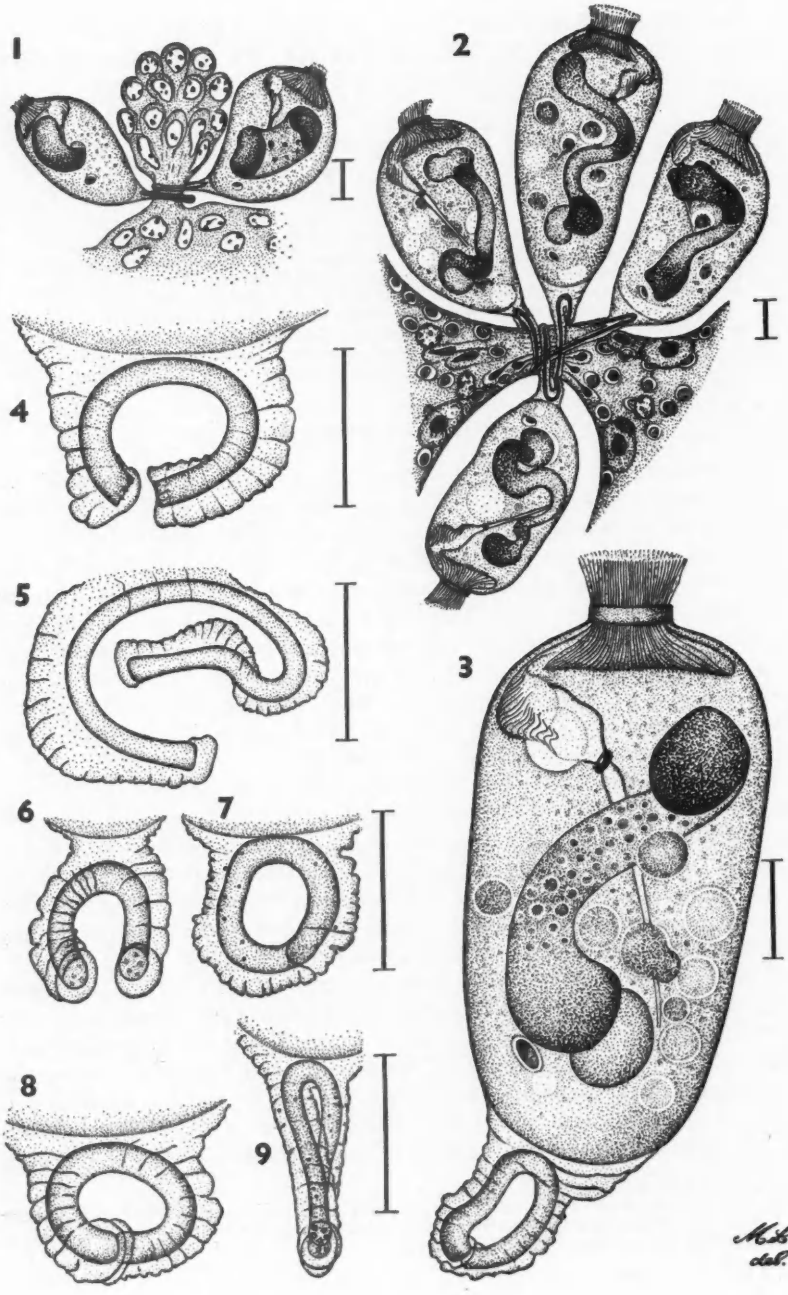
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suggested (6) that *Ellobiophrya* may be descended from an ancestor of the typical *Scyphidia* kind (in which the zooid itself is sharply constricted basally) and *Caliperia* from a *Gerda*-type scyphidian (having a more or less cylindrical zooid, the endoplasm of which is demarcated from a broadly based scopula). The button-like interlocking device of *Ellobiophrya* and the endoskeletal organelle of *Caliperia* are viewed as homologous with the scopular rodlets of the appropriate type of ancestral scyphidian.

A second species of *Caliperia* came to light in 1958 during studies of the parasites of marine animals undertaken at the Fisheries Research Board Biological Station, Saint Andrews, New Brunswick.

Methods and Material

Between June 17 and July 7, 1958, various marine and brackish-water organisms were brought to the laboratory alive and examined for parasites. These animals included 186 fishes of 25 species. As a routine measure, fresh saline preparations from the gills were covered with cover slips and examined by phase contrast. Very numerous examples of the ciliate forming the subject of this paper were discovered in slides from two adults of the common skate, or tobacco box, *Raja erinacea* Mitchill, trawled at 20 fathoms off Minister Island, adjacent to Saint Andrews, Passamaquoddy Bay, on June 19 and 24. All the other fishes, including an example of the starry or thorny skate, *Raja scabrata* Garman, proved negative for the organism, which is described hereunder. Permanent haematoxylin-stained mounts were made, as recently described in this journal (7). All dimensions are derived from the measurement of 50 consecutive examples, except where otherwise stated.

Caliperia brevipes n. sp. (Figs. 1–9)

Habit

Microscopic examination of pieces snipped from the gills revealed great numbers of peritrichous ciliates massed on and about the free edges of the filaments. In life these project into the cavity of a gill pouch, where their epibionts would be exposed to the full force of the branchial current. The ciliates were so tightly pressed together, the tapering posterior extremities of groups of up to 16 individuals being directed in towards a common center, that their real nature could not be determined *in situ*. Apart from the facts that they were relatively sluggish and did not expand the peristome, they bore considerable resemblance to *Epistylis* colonies.

FIGS. 1–9. *Caliperia brevipes* n. sp. All figures prepared with the aid of a Zeiss drawing apparatus, from haematoxylin-stained slides. The scale lines to the right of the figures represent 10 μ at the same magnification.

FIGS. 1 and 2. Habit. × 580.

FIG. 3. Whole animal, detached from host. ×1450.

FIGS. 4–9. The attachment organelle. ×2340.

When teased-up fragments of gill filaments are studied, though, it is seen that each zooid is quite independent of its neighbors and is secured in position by means of the distinctive attachment organelle that characterizes the genus *Caliperia*. The pseudo-colonial appearance of a heavy infestation is due to the frequent use of a single anchorage by two or more individuals. In some instances (Fig. 1) the point of attachment is the base of a bud-like outgrowth of the layer of cells forming the effective membrane across which gaseous exchange takes place in respiration (3), while in others (Fig. 2) it is one of the strands of the superficial capillary network. Elongation of the nuclei of epithelial cells near the attachment organelle (Fig. 1), and the greatly stretched dumbbell-shaped nuclei of erythrocytes in the most constricted parts of capillaries (Fig. 2), are evidence of the tightness of the grip of the ciliate's two caudal processes.

Morphology

Fresh examples examined in saline exhibit little activity. They are urn-shaped and widest anteriorly, the cilia protruding as a sheaf through an opening having a raised rim and corresponding to the neck of the urn. Phase contrast reveals the elongate macronucleus and numerous more or less spherical food inclusions. Under dry objectives the pellicle appears smooth (annuli were not searched for by oil immersion). Ten ciliates comprising a group which a piece of host tissue had protected from compression by the cover slip measured from 64.8 μ to 81.1 μ by 28.8 μ to 41.8 μ.

Examples in permanent mounts exhibit shrinkage due to fixation. They are also flattened during the smearing process, and this renders them relatively broader than in life. Otherwise, as in *C. longipes* (6), the contours of the zooid are little altered, since marked retraction does not take place. Forty (83.3%) of 48 extended ciliates were broadest anteriorly, while 18 (81.8%) of 22 slightly contracted ones were broadest medially. The posterior third was never broader than the remainder of the zooid. The body proper measures from 29.2 to 72.9 μ by 21.3 to 37.5 μ (av., 49.3 by 28.2 μ). Mounted zooids of *C. longipes* are of similar length but relatively broader (av., 51.5 by 38.8 μ). The caudal processes, ranging from 3.6 to 28.6 μ (av., 12.7 μ) in length by from 1.3 to 5.2 μ (av., 3.0 μ) in breadth, are much shorter and appreciably narrower than those of *C. longipes*, which measure from 37.5 to 155.6 μ (av., 90 μ) by 5 to 6 μ.

The siderophilous rim at the entrance to the peristome (Fig. 3) measures from 6.2 to 11.3 μ (av., 8.3 μ) in diameter. General details of the cilia, peristome, vestibule, and contractile vacuole are as in the genotype, from which the skate epibiont differs, however, in the more intense staining reaction of the walls of the vestibule and in having a prominent siderophilous sphincter (Fig. 3) at the cytostome. As in *C. longipes* the cytopharynx is a long tube discharging near the posterior part of the macronucleus. This structure is long and sinuous, measuring from 23.9 to 78.1 μ (av., 50.7 μ) in length and from 2.1 to 12.9 μ (av., 6.5 μ) in breadth. Like that of the genotype it exhibits

numerous chromatin spherules each surrounded by a clear halo. The oval to lenticular micronucleus measures some 4.5 by 2.4 μ and is rather larger than that of the New Zealand species, from which it differs radically in its posterior instead of anterior location.

Stretched to their fullest extent around a support, the caudal processes appear somewhat flattened and strap-like. When torn free during smearing, though, they are relaxed, superficially wrinkled, and round to oval in cross-section (Figs. 3–9). As is seen from the accompanying illustrations, the endoskeletal rod is always at the inner margin. This serves as an indication of the structure's elasticity, as does a figure-of-eight shape that the organelle sometimes assumes. Siderophilous granules may be present (Figs. 7, 9), but are less evident than in *C. longipes*. Closure of the attachment organelle is effected by inward pressure as in the latter species, the pellicle of the two processes sometimes being bulged and flattened at the point of contact (Fig. 8). Although the extremities of the endoskeletal rod appear somewhat rugose in favorably orientated specimens (Figs. 4, 6, 9), there is no locking device as in *Ellobiophrya*. Damage in smearing merely results in terminal contact being broken, each process remaining intact.

This epibiont from *Raja erinacea* is clearly referable to the genus *Caliperia*. Differing as it does from the only species previously described in its much shorter caudal processes, the posterior location of the micronucleus, the presence of a prominent siderophilous sphincter at the cytostome, and in other respects as detailed herein, it is designated *Caliperia brevipes* n. sp. in reference to the first and most obvious of these features. The type slide is in my collection.

Discussion

Caliperia brevipes resembles *Ellobiophrya donacis* in the relative length of the caudal processes and the presence of a siderophilous sphincter at the cytostome. Nevertheless, differences of at least generic significance still separate *Caliperia* from the lamellibranch epibiont. The latter is basally, not anteriorly, widest, and the endoplasm of the zooid is continuous with that of the caudal processes. These altogether lack an endoskeleton, but instead have a terminal locking organelle so efficient that under stress the attachment ring usually tears apart at some other point. Another feature peculiar to *E. donacis* is the elliptical instead of elongate shape of the macronucleus in its normal resting state (2).

E. donacis, as described and figured by Chatton and Lwoff (1, 2), hangs loosely, like a padlock, from a filament of the gill grid of the host. Its adaptations are excellently suited to this purpose, just as are those of the two species of *Caliperia* to attachment to the gills of fishes. All three ciliates obtain their food from the water streaming over the branchiae of the host. It is obviously advantageous for *Ellobiophrya* to be able to pivot about the anchorage to feed on either side of the gill grid and to conform to the lamellibranch's reversals of direction of the branchial current. Such reversals for

the purpose of clearing blockages occur in skates too from time to time (3), but a temporary change in the direction of flow does not necessitate any reorientation of a *Caliperia* projecting into the cavity of a gill pouch. The appropriate attachment organelle in this case is one which will ensure tightness of grip.

It should be noted that these highly interesting ciliates have all been described from bottom-dwelling littoral animals, albeit from widely separated localities. Further studies may reveal their more extensive geographical distribution, and are in any case required before it can be determined whether the resemblances between *Ellobiophrya* and *Caliperia* are due to convergence operating upon the products of the two distinct scyphidian stems—as is suggested by the fact that the endoplasm of the zooid is continuous with that of the caudal processes in the former genus but not in the latter one—or to descent from a common stock otherwise obscured by specialized adaptations to very different hosts.

Acknowledgments

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THE EFFECT OF MICROENVIRONMENT ON THE FREE-LIVING STAGES OF BUNOSTOMUM TRIGONOCEPHALUM[1]

EDWARD A. BELLE[2]

Abstract

This investigation has shown that temperature and relative humidity are the microenvironmental factors governing the development of the free-living stages of *Bunostomum trigonocephalum*.

The geographical distribution of nematode parasites is determined by various factors of which the most important are the effects of the microenvironment on those stages which occur outside of the host and serve to convey the parasite from one animal to another.

One of the more important pathogens of sheep is the so-called hookworm, *Bunostomum trigonocephalum* Rud. (synonyms *Uncinaria cernua* Crep. and *Monodontus trigonocephalus* (Railliet)). This worm was studied by Baillet (1), who noted that the eggs could develop and hatch in water within 4 to 6 days. It was studied further by Hesse (5), Cameron (2, 3), and Ortlepp (9), but no carefully controlled observations on its biology in the free state have so far been published. Accordingly, this investigation was undertaken to determine accurately the effects of temperature and humidity on the free stages.

Worms were collected from the intestines of sheep obtained from Montreal abattoirs. The females were ground up in a mortar with a small quantity of water and the suspension passed through four layers of cheesecloth. Cultures were made from the eggs thus obtained by adding the eggs to sterilized sheep faeces mixed with one-third part of vegetable charcoal and some sterilized sand. Control cultures were invariably negative for nematode larvae but permitted the growth of bacteria.

Except for a specially designed microclimate cabinet, standard laboratory equipment was used throughout the experiments. The special cabinet was maintained at 5°.* It contained six small chambers which could be held at specified temperatures under room temperature but above 5°. Each chamber contained a fan to maintain an even temperature, and a stainless steel container for the chemical solutions necessary to maintain a specific relative humidity (R.H.). These solutions were prepared from the list published by O'Brien (8). The cabinet itself was kept in a constant temperature room.

Ova

The Range of Temperature Necessary for Development

Cultures, each containing 2000 eggs, were incubated at temperatures from 10 to 40°. After the 2nd day, one culture was removed daily and the larvae recovered by either the Baermann technique or that outlined by Cameron (2).

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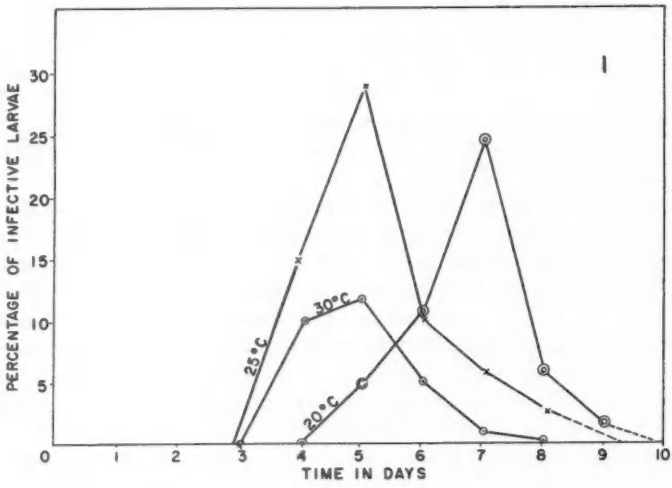
*Temperatures referred to throughout this paper are in degrees Celsius (centigrade).



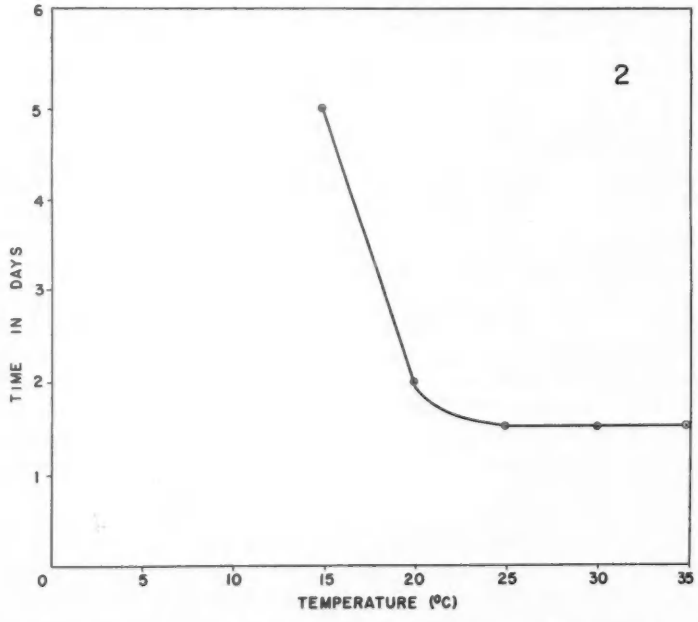


FIG. 1. Optimum development temperature of eggs of *B. trigonocephalum*.
FIG. 2. Percentage of larvae hatching at different temperatures.

The number of larvae recovered was ascertained by a simple dilution method. The average number was computed by dividing the sum total of the larvae collected at each specified temperature by the number of times the experiment was conducted at that temperature. In order to determine the number of larvae for a given day, the amount recovered for the previous day (or days) was subtracted from the total amount recovered for the whole period. Thus, at 25°, 45% of the eggs developed to infective larvae by the 5th day, but 15% of these had already developed on the 4th day. Therefore, 30% actually reached the infective stage *on* the 5th day. Table I summarizes the results obtained at temperatures from 15 to 35°. Figure 1 shows the optimum development temperature.

TABLE I

Effect of different temperatures on the development of *B. trigonocephalum* eggs to the infective larval stage

Incubation period, days	Temperature									
	15°		20°		25°		30°		35°	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
4	–	–	–	–	300	15	200	10	60	3
5	–	–	100	5	600	30	240	12	100	5
6	–	–	220	11	200	10	100	5	40	2
7	–	–	500	25	120	6	20	1	–	–
8	60	3	120	6	60	3	–	–	–	–
9	100	5	40	2	–	–	–	–	–	–
10	40	2	–	–	–	–	–	–	–	–

NOTE: *a*—average number of larvae recovered.
b—percentage of larvae recovered.

The Effect of High and Low Temperatures on Development

After the range in temperature at which complete development took place had been determined, it was necessary to determine to what extent development was hindered by extremes in temperature; below 15° was considered to be low, and above 35° was considered as high.

Eggs were kept in cultures at 40° and 100% R.H. for periods varying from 3 to 24 hours, then transferred to 25° for 5 days. The results showed that after 3 hours' exposure, 10% of the 600 eggs used in each culture developed to the infective stage, after 6 hours, 6%. None of the eggs survived for longer than 12 hours.

Eggs in cultures were exposed to low temperatures between −12 and +5° for varying periods, then transferred to the optimum temperature. The results shown in Table II are expressed as a percentage of the number recovered under optimum conditions.

Eggs in cultures were kept at 0° for 12 hours then transferred immediately to 25° for 12 hours. This procedure was repeated for 6 days before the material was baermanned. The results showed that 10% of the eggs developed to infective larvae. Alternating the temperature of culture between 5 and 25° had no effect, whereas alternating it between −5 and 25° prevented development. In order to determine whether the eggs died or whether

development was only inhibited temporarily, some cultures, after being subjected to alternate freezing (−5°) and thawing (25°), were kept continuously at 25° for an additional 6 days. As no larvae were recovered, it was concluded that the eggs died during the periods of freezing and thawing.

TABLE II

Effect of low temperatures on the viability of the eggs of *B. trigonocephalum* in faecal cultures

5°		0°		−5°		−12°	
Time, weeks	% visible	Time, days	% visible	Time, days	% visible	Time, hours	% visible
8	25	7	25	4	35	1	15
10	12	14	15	5	20	2	0
12	4	18	0	–	–	–	–

Effect of Temperature on Embryonation

In these experiments, in order to observe the embryonation process more closely, it was necessary to culture the eggs in plain water. A known number of eggs was placed in a number of small watch glasses containing very little water. The watch glasses were kept at different temperatures and observations made frequently. The results obtained are shown in Table III, and plotted in Fig. 2.

TABLE III

Embryonation and hatching at different temperatures

Temperature, 0°	% embryonated	Time	% hatched	Time embryonating and hatching
15	15	3 days	5	5 days
20	40	36 hours	10	48 hours
25	50	25 hours	15	36 hours
30	40	24 hours	10	36 hours
35	16	24 hours	2	36 hours

It is known that embryonation of the eggs of various species of nematodes is inhibited at lower temperatures, the degree of inhibition depending on the species. Eggs of *B. trigonocephalum* were kept at 0° for 4 days, then allowed to thaw at 25° and kept at this temperature for 2 days; 15% of the eggs hatched. Under normal conditions, these eggs would have hatched in 36 hours as was the case in the control samples. When another lot of eggs was frozen at 0° for 7 days, then transferred to 25°, they took 5 days to embryonate and hatch. The eggs died when frozen at 0° for 14 days. Eggs kept at 5° for 14 days took 3 days to embryonate and hatch when transferred to optimum conditions. At −12° and −17° for 1 hour, 15% of the eggs embryonated and hatched within 3 days when transferred to 25°. They died when kept at these temperatures for 2 hours.

The Effect of Relative Humidity on Development

The development of the eggs and subsequent survival of the larvae depend considerably on the relative humidity of their environment. For specific tests, the humidity has to be rigidly controlled, and this was done by the use of accurately tested salt solutions (8).

A culture of sand, faeces, and charcoal was prepared and a few grams placed in small, uncovered Petri dishes. Six hundred *Bunostomum* eggs were then pipetted, with as little water as possible, on to each culture. The cultures were then placed in chambers in the cabinet set at 25° and in which specific salt solutions were placed in each to achieve a R.H. of 20, 44, 66, 81, and 92%. Tap water was used instead of salt solution to obtain a R.H. of 100%. Five cultures were used in each test. Those set at 81, 92, and 100% R.H. were slightly moistened in order to attain the required humidity quickly. The cultures were exposed for 14 days and then baermanned. The results are shown in Table IV.

TABLE IV

Effects of relative humidity on the development of the egg

% R.H.	Results
20	No larvae were collected after the culture had been allowed to baermann for 24 hours
44	As above
66	5% preinfective larvae collected after baermanning for 24 hours
81	7% infective larvae collected after baermanning for 2 hours; no larvae collected after baermanning for 24 hours
92	10% infective larvae collected after baermanning for 2 hours
100	30% infective larvae collected

Another set of cultures was then exposed for 7 days at the R.H.'s mentioned above. These were then moistened and kept at 100% R.H. for a further 7 days. The results showed that in the cultures originally kept at 44% R.H. there was no development whereas at 66 to 92% R.H. development was normal. The negative results obtained at 44% R H. may be due to two reasons: the eggs may have died within 7 days, or, they may have hatched and the preinfective larvae died. The first reason is probably the correct one in that it is unlikely that eggs would hatch at 44% R.H. and not at 66% R.H.

Further experiments were carried out to determine how long eggs would remain viable at 44% R.H. Several cultures were set up, one being removed each day and baermanned immediately, and another removed, moistened, and kept at 25° for 7 days. After the 1st day, the number of larvae recovered became progressively fewer. On the 9th to 7th days, no larvae were recovered from either culture. It must be concluded, therefore, that the eggs remained viable at 44% R.H. for 6 to 7 days after which time they deteriorated. All the larvae died after 5 days' exposure at 25% R.H. More than half the larvae survived at 66% R.H. for 15 days.

The Effect of pH

The results obtained on the effect of pH are shown in Fig. 3. In regulating the pH, solutions of sodium bicarbonate, sodium carbonate, potassium chloride, and calcium phosphate were used. None of these solutions, even in concentrations stronger than those used to regulate the pH, had any direct effect on the eggs. As formalin in a very weak solution (1:1000) did not kill the eggs, I believe that any lethal effect in greater concentrations was due to its acidity. This solution was, therefore, used for the high acidity range in the pH experiments. In all respects, the chemical disinfectants were more lethal to the eggs than were the chemical fertilizers.

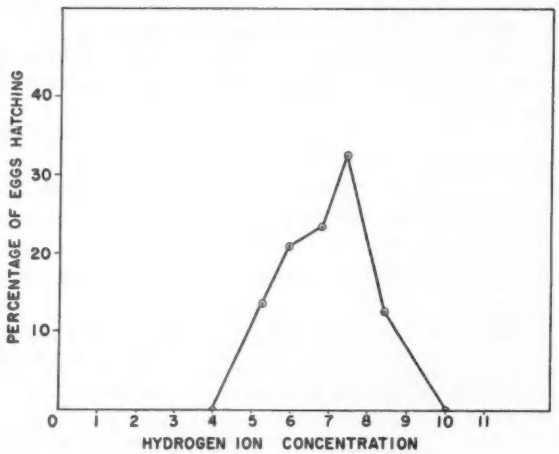


FIG. 3. Effect of pH on hatching of eggs.

The influence of acidity on the development and hatching of the eggs of parasitic nematodes has not been clearly understood. Looss (7) found that laboratory cultures of *Ancylostoma duodenale* did not develop well. He attributed this to the accumulation of decomposed organic products, particularly various acids. By adding animal charcoal to the culture, there was a considerable increase in the number of eggs that hatched. Looss claimed that calcium carbonate added to cultures "fixed certain acids" and thus proved useful. Stoll (12) found that the unmodified faeces (pH 4.5–5.0) of patients infected with hookworm in Puerto Rico gave a poor yield of larvae, and concluded that the failure of the eggs to hatch was due to the low acidity. His results are comparable with mine. Development occurred at a range of 5.2 to 8.4, although the most suitable pH was between 6 and 7.5.

Preinfective Larvae

The preinfective larvae of *B. trigonocephalum* are entirely free-feeding and free-living, and so may be exposed to variations of temperature and relative

humidity. Experiments were carried out to determine their reactions to these factors.

Preinfective larvae were collected from faecal cultures 2 to 3 days old. These larvae, having been nourished, were more healthy than those hatched in plain water.

At temperatures ranging between −12 and −17° preinfective larvae died within an hour. At 0°, all survived for 7 days, and 5% for 15 days. When the temperature was raised to 5° they molted to infective larvae. They died within a few hours at 40°.

At relative humidities lower than 66% at room temperature, the larvae died within a day.

These results show that the preinfective larvae are short-lived. It is believed that they do not remain dormant. If conditions are unfavorable for their development, they have no means of counteracting them, and consequently die relatively quickly as compared with the eggs or infective larvae. If they are not killed within a few days they develop to the infective stage, as was found at temperatures from 5 to 35°. Schwartz (10) found that at 8° for 10 days many of the preinfective larvae of *B. phlebotomum* degenerated. I have found that larvae of the related *B. trigonocephalum* develop to the infective stage when kept at 5° for 2 to 3 weeks.

Infective Larvae

A known number of infective larvae was placed in separate beakers containing water. The same number of larvae was placed on moist filter paper which was then carefully folded and inserted into a vial which was placed in a sealed jar containing some water. This ensured a R.H. of 100% throughout the experiment. The cultures were kept at constant temperatures between 10 and 30° and examined once weekly. Cultures kept at 35° were examined every 3 days.

The larvae lived for 12 weeks at temperatures between 10 and 25°. At the 18th week all the larvae kept at between 10 and 20° were still alive and active, but at 25° there was only an 80% survival of those kept in water; unfortunately, the filter paper containing the larvae at this temperature dried up after the 9th week and, therefore, these cultures were discontinued.

At 30°, 75% of the larvae survived for 8 weeks and 50% survived for 12 weeks. The larvae became inactive at 35° after 34 days; they were then transferred to 20° but did not recover. Larvae kept on moist filter paper at 35° for 38 days showed sluggish movements when they were placed in water at 25°. At 40° in water, there was a 47% survival for 3 days. While they were very active, all died when kept at this temperature for 6 days. Six hours at 45° was fatal to the larvae.

Effect of Low Temperatures on Survival

Infective larvae survived for over 4 months at 5°. When transferred to room temperature they became very active after a few minutes. After 6 days

at 0° they were slightly coiled but when removed to 25° they uncoiled after an hour and moved sluggishly; 3 hours later they were quite active. Larvae exposed to 0° for 12 days showed the same coiling feature but only 40% of them survived, while none survived when kept at this temperature for 15 days.

When the larvae were kept at −12° for 1 hour, they became tightly coiled like a compressed watch spring. After thawing for 2 hours at 25° they uncoiled and became as active as the control group. After 90 minutes' exposure, however, they uncoiled and died shortly afterwards. A few larvae survived for half-an-hour at −17 to −20° but when transferred to room temperature all died within a week.

Ethyl alcohol and dry ice were used to quick-freeze the larvae at temperatures below −20°. The larvae were kept in a test tube which was suspended in a beaker containing a mixture of 95% ethyl alcohol and dry ice. When the desired temperature was reached, the tube containing the larvae was placed in the mixture. Larvae frozen at −25° for 5 minutes were allowed to thaw rapidly at 37.5°. Another set of larvae were thawed out slowly at 25°. None showed signs of recovery.

In other experiments, the larvae were "conditioned" before being subjected to the low temperatures; that is, they were placed at 10, 5, 0, −5, and finally at −12°. They were kept at each temperature for 30 minutes with no time interval elapsing between any two temperatures. Within 3 hours of the last exposure, there was 65% mortality.

Effect of Relative Humidity

The infective larvae were placed on filter paper or in faecal cultures and then kept in humidity chambers set at 23 to 25°, with relative humidities ranging from 20 to 100%. An estimated 600 larvae were used for each experiment. The results showed that at 20 and 44% R.H. 32 and 38%, respectively, survived for 12 hours but none lived for 24 hours. At 66% R.H. there was 12% survival for 24 hours, but all died in 2 days. After 9 days at 81% R.H. 25% survived, and at 92% R.H., there was 34% survival. Nearly all the larvae survived at 100% R.H.

Discussion

No development of the eggs of *B. trigonocephalum* took place at temperatures under 15° or above 35°. Optimum development was reached on the 5th day and the most suitable temperatures were between 20 and 30°.

Freezing temperatures and those of 40° and above were harmful to the eggs; at 0° they remained viable for only 2 weeks. This partly explains why the adult worm is uncommon in tropical regions and in those that remain frozen for the greater part of the year. However, in Canada and other countries with prolonged periods of freezing temperatures, sheep are usually kept housed, so that the parasite has no difficulty in overwintering. Alternate freezing and thawing was injurious to the eggs, less than 10% reaching the infective stage when alternately frozen for 6 days at 0° and thawed at 25°,

allowing 12 hours at each temperature. When eggs were kept constantly at 0° for 7 days, then transferred to 25° for an additional 6 days, 25% developed into infective larvae. Preinfective larvae remained alive at 0° for 14 days. In a few cases, the eggs were recovered from the culture and a few had developed to the early tadpole stage. Apparently, this stage is less resistant than the unembryonated egg and than the preinfective larvae.

Observations on embryonation showed that while 50% of the eggs embryonated in plain water at 25°, only 15% hatched. Very few reached the infective stage but larvae were frequently observed within the egg actively trying to escape. When the eggs were incubated in faecal cultures, the percentage that embryonated and hatched was considerably higher. I believe, therefore, that to obtain maximum hatching, there has to be some form of resistance against which the eggs must push. This assists in the rupture of the outer membrane. These results differ from those of Hesse (5), who found that eggs of *B. trigonocephalum* hatched at 22° in plain water after 24 hours and developed to the infective stage within 4 days.

Schwartz (10) found that eggs of the cattle hookworm, *Bunostomum phlebotomum*, took 96 hours to hatch at from 21 to 27°. Sprent (11) found that eggs of the same species began to hatch in 24 hours, although many hours passed while the embryo moved spasmodically within the shell. Sprent also stated, quoting Conradi and Barnett (1908), that the eggs of *B. phlebotomum* took 31 days to hatch at from 10 to 15°. My results differ from those of Schwartz. I found that the eggs of *B. trigonocephalum* did not hatch at 10°, that they took 5 days to embryonate and hatch at 15°, and hatched in less than 48 hours at from 21 to 27°.

The humidity requirements for the eggs show that although they are unable to withstand desiccation for long (less than 7 days at 44% R.H.) nevertheless the amount of moisture required for complete development is only 81% at 25°. I found that the lower the temperature the less the amount of moisture required for development.

The duration of the life of the infective larvae under optimum conditions is not known. In clear water they live for several months. A temperature of 10–20° is most suitable for their existence. Larvae recovered from faecal cultures lived considerably longer than those which developed in plain water. In the latter case, of course, there is little food on which the second preinfective stage can feed. Infective larvae incubated in plain water showed a 15% survival rate at 25° in 15 days, while those grown in a culture of faeces and charcoal showed an 80% survival on the 12th week.

Kates (6) stated that the preparasitic stages of *B. trigonocephalum* showed no evidence of survival over the winter months on pastures in Maryland, U.S.A. The temperatures he recorded for January and February were between −5 and +5°. In the usual absence of a snow cover in Maryland, this range in temperature probably represents an approximation to the actual temperature encountered by the larvae. My investigation has shown that all the larvae of *B. trigonocephalum* died within 15 days at 0°.

During a period of freezing, certain morphological and physiological changes take place in the infective larvae. They are invariably shrivelled and coiled in subfreezing temperatures. When subjected to quick deep-freezing at temperatures below $-25°$ the intestine ruptures and the contents escape.

The resistance of a given organism to desiccation is to some extent determined by the rapidity of the drying agent but it also depends on the temperature at which drying takes place. Nematode larvae are naturally aquatic; they have no means of storing excess water and when exposed to the atmosphere, die within a short time unless the relative humidity is high. It is often claimed that the larvae could withstand drought (or desiccation in the laboratory) for a long time. The humidity in actual contact with the larvae in an open pasture is generally higher than that in the ambient atmosphere. Crofton (4) found that between grass blades, the almost closed system usually has a different moisture content from that of the outside air. He made several observations and found that even during a prolonged drought of more than 3 weeks, the relative humidity on normal, well-drained pastures, within the 'mat' was above 90%. The humidity at the tips of grass blades was less than that at the base by as much as 20%. Under these conditions, it can be readily understood why in apparently dry seasons, infective larvae of nematodes can remain alive for a few weeks.

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GEOGRAPHICAL DISTRIBUTION AND VARIATION OF THE SACRAMENTO MOUNTAINS SALAMANDER, ANEIDES HARDII[1]

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Abstract

The Sacramento Mountains salamander, *Aneides hardii*, previously known only from the region of the type locality (Sacramento Mountains, New Mexico) is reported from the adjoining White and Capitan Mountains. The three populations of *A. hardii* are believed to be geographically disjunct. A study of the differences between the three populations did not indicate that subspecific recognition is warranted.

The purposes of this report are to call attention to the occurrence of *Aneides hardii* in the Capitan Mountains and the White Mountains (Sierra Blanca), Lincoln County, New Mexico, and to indicate briefly the variation found to exist between these populations. *A. hardii* has previously been reported only from the Sacramento Mountains, Otero County, New Mexico (3).

Sites and dates for the collections made are as follows: (1) Capitan Mountains, elevation 9600 ft, 0.5 miles north of Summit Spring, August 6 and 9, 1958; also at 9300 ft, 1.25 miles southeast of Koprian Springs, August 9, 1958; (2) White Mountains, elevation approximately 9000 ft, 1.5 miles southwest of Monjeau Lookout, Lincoln National Forest, August 10, 1958; (3) Sacramento Mountains, elevation 9150 ft, Wofford Lookout, Lincoln National Forest, August 16, 1958; also at 8600 ft, 6 miles northeast of Cloudcroft, August 7 and 10, 1958.

A study of the natural history of *Aneides hardii*, based in part on these samples, is in the press (1). A list of the institutions at which specimens have been deposited is included therein.

Habitat

Our experience indicates that *A. hardii* is restricted mainly to the cooler and more humid, north and east-facing slopes of the Canadian Zone. On the Capitan, White, and Sacramento Mountains, this zone is a narrow, irregular, and broken area that attains its full vertical expression only on Sierra Blanca. The collection sites were characterized by a forest of spruce, fir, and aspen with an understory of Rocky Mountain maple, ocean spray (*Holodiscus dumosus*), cliffbush (*Jamesia americana*), *Ribes* spp., cinquefoil, geranium, Arizona fescue, wild oat grass, and redtop (*Agrostis idahoensis*).

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TABLE I

Measurements of adult *Aneides hardii* from the Capitan, Sacramento, and White Mountains of New Mexico (measurements to nearest mm)

	Capitan Mountains				Sacramento Mountains		White Mountains	
	Adults = S-V >40 mm		Adults = S-V >43 mm		Adults = S-V >40 mm		Adults = S-V >40 mm	
	Males	Females	Males	Females	Males	Females	Males	Females
Snout-vent length								
Number measured	35	22	30	18	15	13	22	7
Range	40–55	40–53	44–55	45–53	42–50	40–48	40–54	40–47
Mean	49.51	47.09	50.93	48.61	47.07	43.23	44.68	42.57
	±0.73	±0.88	±0.49	±0.65	±0.68	±0.66	±0.69	±0.95
Standard deviation	4.33	4.13	2.73	2.74	2.63	2.38	3.23	2.51
Tail length								
Number measured	34	21	30	17	15	13	21	7
Range	34–55	31–46	39–55	40–46	40–52	36–44	37–55	36–42
Mean	46.91	40.81	48.17	42.35	46.60	39.08	43.38	38.71
	±0.88	±0.78	±0.69	±0.39	±0.96	±0.71	±0.99	±1.04
Standard deviation	5.14	3.67	3.76	1.62	3.72	2.57	4.54	2.74
Snout-vent/tail length								
Number measured	34	21	30	17	15	13	21	7
Range	0.95–1.21	1.05–1.32	0.98–1.18	1.05–1.27	0.92–1.12	1.02–1.19	0.86–1.19	1.00–1.18
Mean	1.07	1.16	1.06	1.15	1.01	1.11	1.03	1.10
	±0.01	±0.05	±0.01	±0.02	±0.01	±0.01	±0.02	±0.02
Standard deviation	0.06	0.23	0.05	0.07	0.05	0.05	0.09	0.06

Geographical Considerations

The authors believe that the *Aneides* populations inhabiting the Capitan, White, and Sacramento Mountains are geographically disjunct. However, the mountains themselves form a continuous highland area in southeastern New Mexico, rising above the surrounding desert to a maximum height of above 12,000 ft on Sierra Blanca Peak of the White Mountains. Of the three populations of *A. hardii* under consideration, that of the Capitan Mountains is the most clearly separate geographically. Habitats suitable for *A. hardii* do not exist in the area between the Capitan and White Mountains, the intervening upland region being one of piñon–juniper vegetation. The straight line distance between Sierra Blanca and Capitan Mountain is 12 miles. Thus there is no question as to the isolation of the Capitan population.

Discontinuity of appropriate habitats for *A. hardii* is not as obvious in the case of the White and Sacramento Mountains, in which areas of suitable spruce–fir vegetation approach within 2 to 3 miles of each other where the two ranges join. There is, in fact, a narrow connection of mixed ponderosa pine – spruce – fir. The critical area occurs at an elevation of about 7500 ft along U.S. Highway 70 just east of the pass (Apache Summit) between the two ranges. Salamanders have been sought at this and similar localities extending down from the White Mountains without success, and we believe, therefore, that *A. hardii* is confined to the higher slopes and does not extend down along tongues of spruce–fir to where the latter become extensively mixed with ponderosa pine. If this is true, then it follows that the Sacramento and White Mountains populations are also disjunct.

Variation

Our collections consisted of 72 specimens from the Capitan Mountains, 52 from the Sacramentos, and 53 from the White Mountains. Of these, 57, 28, and 29, respectively, were considered adult and it is on these that the following discussion is based. The separation of adult from juvenile animals was made at 40 mm snout–vent length (1). Measurements were made of: snout–vent length and tail length (Table I), snout to gular fold, width of head, and length of fore and hind limbs. The number of costal grooves and of costal folds between the tips of the adpressed limbs were counted. Teeth were not counted. The pigmentation of each specimen was noted.

Inspection of the data and of the pigmentation of the specimens themselves suggested that the major differences between the samples would be in snout–vent length and in the degree of guanophore coverage of the gular area. Analysis of variance showed that statistically significant differences existed between some of the collections in these characters. The mean snout–vent length was greater in the Capitan sample than in either the Sacramento (males $P = 0.05$; females $P = 0.01$) or the White Mountain ($P = 0.01$),

but no difference was found between the latter groups. With the exception of tail length, the other measurements within each sample paralleled those for snout–vent length and, therefore, were not analyzed statistically. Although the specimens of the Capitan sample had longer bodies, the tails were relatively shorter. The ratio of snout–vent length to tail length was significantly greater for the Capitan sample than for the Sacramento (males $P = 0.01$; females $P = 0.05$) or for the White Mountain samples ($P = 0.05$). The latter groups did not differ significantly from each other. In females, the tail length never exceeded the snout–vent length. In males from the Sacramento and White Mountains, however, the tail was longer than the body in 33% and 29% of the specimens, respectively, while in the Capitan sample 6% of the males had a tail which exceeded the body in length.

Numerical values were assigned to each specimen on the basis of guanophore coverage of the gular area. For males, these values were found to be significantly less ($P = 0.01$) in the Sacramento specimens than in either the White or Capitan Mountains samples. The latter, however, did not differ from each other, nor were statistically significant differences found between adult female samples.

Although some of the population means for the characters, snout–vent length, snout–vent length/tail length, and guanophore coverage of the gular area differed significantly, individuals could not be separated because the overlap in these characters is considerable. Coefficients of the difference (2) were calculated to determine the extent of non-overlap in these characters, but all values thus obtained fell below the level usually regarded as indicating subspecific distinctness. The greatest joint non-overlap (80%; coefficient of the difference = 0.85) was found in the comparison of the snout–vent lengths of males from the Capitan and White Mountains. However, as the Capitan specimens are larger, it is not unreasonable to assume that they would reach adulthood at a larger size than the others. The frequency distribution of the snout–vent length of the entire Capitan sample suggested that a more realistic separation of juveniles from adults might be made at 43 mm than at 40 mm. When the means and standard deviations were recalculated for the Capitan sample eliminating those smaller individuals that might introduce bias (Table I), it was found that the joint non-overlap (85%) was still insufficient to consider the Capitan population subspecifically distinct.

Thus, the more geographically isolated population of *Aneides hardii* inhabiting the Capitan Mountains is morphologically the most distinct, the difference between the other two populations, those of the Sacramento and White Mountains, being less marked. Though the latter are geographically isolated at present, the barrier is presumably weak and it seems probable that relatively small climatic changes would suffice to reunite the two populations. Subspecific recognition does not appear to be warranted for any of the populations on the basis of the characters studied.

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THE EFFECTS OF TEMPERATURE AND HUMIDITY ON THE FREE-LIVING STAGES OF TRICHOSTRONGYLUS RETORTAEFORMIS[1]

DEVANDRA PRASAD[2]

Abstract

The optimum temperature for development of *Trichostrongylus retortaeformis* is about 25° C in a wet faecal culture, when the infective stage is reached in from 3 to 5 days, but at 3 to 5° C a few larvae can develop in 8 to 10 weeks, and infective larvae can survive for 13 weeks. Both eggs and larvae can survive desiccation for considerable periods.

One of the more common nematode parasites of the European rabbit (*Oryctolagus cuniculus*) is *Trichostrongylus retortaeformis*. As the microenvironment surrounds the free stages of this and other nematodes, a study, under carefully controlled laboratory conditions, was made of the effects of temperature and humidity on the eggs, preinfective larvae, and infective larvae of this parasite.

Laboratory-bred young rabbits were infected with *T. retortaeformis* from cultures obtained through the courtesy of Dr. E. L. Taylor, the Veterinary Laboratory, Ministry of Agriculture, Fisheries, and Food, Weybridge, England.

Standard laboratory apparatus and refrigerators were used in this study. The only special equipment consisted of a cabinet containing six identical chambers, each cabinet capable of being set to different combinations of temperature and relative humidity. The cabinet itself was kept in a constant temperature room. In addition, a set of air-tight rectangular wooden humidity chambers were kept at room temperature. By placement of specific salt solutions (3) in these, a range of from 20 to 100% relative humidity (R.H.) was attained.

Whenever possible, parallel studies were made with the free-living stages, eggs, preinfective larvae, and infective larvae. Eggs were obtained from faecal samples within half an hour of being passed. Larvae were obtained by making cultures from fresh faecal samples prepared according to the technique of Whitlock (5) and incubated at 24 to 25°* for 5 to 6 days. Infective larvae were collected in a very clean state either by Whitlock's (5) technique, by a modified Baermann technique (4), or by the regular Baermann technique.

Ova

Effect of Temperature on Hatching

Ova of *T. retortaeformis* were held in 3 ml of water in excavated glass blocks covered by glass lids to prevent excess evaporation and held at a variety of

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*Temperatures referred to throughout this paper are in degrees Celsius (Centigrade).

temperatures. Control samples were held at 24 to 25°. About 150 to 200 eggs were used in each trial. The small number of eggs used facilitated counting and individual observation of each egg.

The results showed that temperatures above the optimum (25°) do not necessarily decrease hatching time. Temperatures below the optimum had a marked effect in increasing hatching time. Ova in water hatched in 5 to 5½ days at 10° and in 9 days at 5°. At 40° none of the ova hatched (Fig. 1).

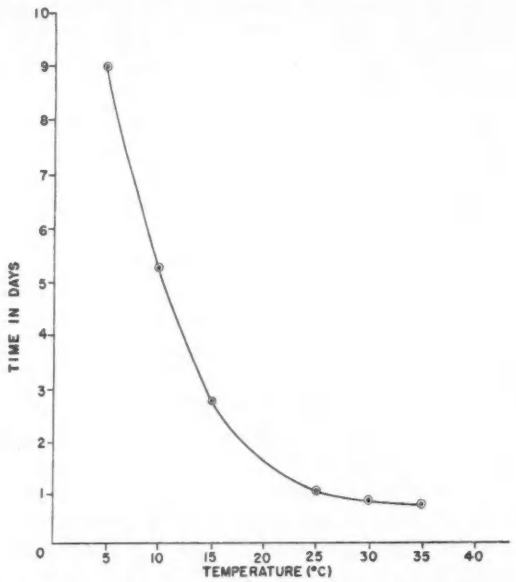

FIG. 1. Hatching time of ova of *T. retortaeformis* at different temperatures.

Optimum Temperature Determination

Fresh rabbit faeces heavily infected with eggs were crushed and mixed and weighed out into almost equal amounts in each of several small Petri dishes (1.5 in. diam.). Simultaneously, two 2-g samples from the same lot of faeces were removed for eggs per gram (e.p.g.) count by the McMaster method (5). Each culture was moistened with about the same quantity of water. The Petri dishes were then placed in Coplin jars which also contained a little water in order to keep the atmosphere inside the jars saturated. The purpose of this step was to obviate differences in relative humidity around the faecal culture. The Coplin jars were sealed tightly with petroleum jelly. Five or six jars were then placed in chambers in the special cabinet maintained at 20, 25, and 30°. After 2 days one culture from each chamber was removed and baermanned immediately. Three hours later 50 ml of bottom fluid was collected from each funnel and the number of larvae estimated by the dilution method. The same procedure was repeated for 5 days. Table I records the

results obtained in these tests. As will be seen, there was a gradual increase in the number of infective larvae recovered. As cultures were removed daily, baermanned, and the recovered larvae counted, it is considered that the excess of larvae obtained on each of the following days over the preceding ones gave the actual number of larvae reaching the infective stage between two consecutive days. For instance, at 25° at the end of 3 days 15.5% infective larvae and at the end of 4 days, 35% infective larvae were recovered. The difference, 19.5%, represents the percentage of infective larvae which reached the infective stage between the 3rd and 4th day, and called "on the 4th day" (Fig. 2). Table II expresses the figures thus obtained. From this it will be seen that 25° is the optimum temperature for the development of larvae to the infective stage.

The infective larvae recovered at the different temperatures were placed in water at 30° to discover their maximum period of survival. The larvae from 30° cultures died in 40 to 45 days, those from 25 and 20° remained alive for more than 75 days.

TABLE I

Number and percentage of infective larvae recovered on different days at different temperatures

Temperature, °C	2 days		3 days		4 days		5 days		6 days	
	Infective larvae	%	Infective larvae	%	Infective larvae	%	Infective larvae	%	Infective larvae	%
20	0	0	240	1.44	1,520	8.5	3,420	19	5,058	28.1
25	0	0	2,800	15.5	6,260	35	9,360	52	10,980	61
30	240	1.33	1,640	9	2,340	13	2,430	13.5	2,358	–

NOTE: Estimated number of ova (based on e.p.g. count) in each culture, approximately 18,000.

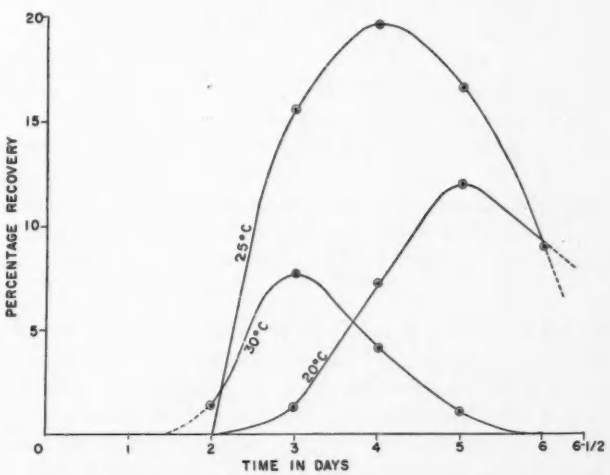

FIG. 2. Percentage recovery of infective larvae of *T. retortaeformis* on different days at different temperatures.

TABLE II

Percentages of infective larvae formed on different days at different temperatures

Temperature, °C	Days				
	2nd	3rd	4th	5th	6th
20	0	1.3	7.17	11.83	9.1
25	0	15.5	19.5	16.5	9
30	1.33	7.67	4	1.1	–

Suboptimum Temperature Determination

Temperatures above and below the optimum at which eggs are able to develop to maturity have been regarded as suboptimum in this study, and the present experiments were designed to determine the *temperature* range above and below optimum at which eggs reach maturity and the *period* at these temperatures in which the maximum number of eggs reach maturity.

Moist faecal cultures containing about 2000 eggs were held at temperatures from 10 to 45° for up to 10 days. Cultures were inspected periodically and moisture added as necessary. At the end of the incubation period the cultures were removed, baermanned, and the larvae recovered. It was found that in these cultures, eggs could complete their development at between 10 and 35°. At either extreme of the temperature range very few eggs were recovered (Fig. 3).

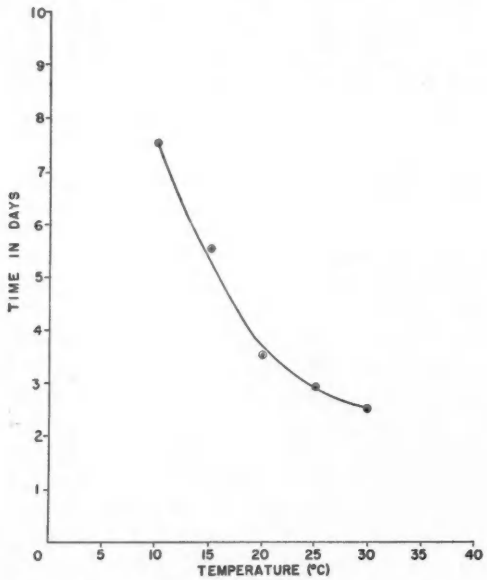

FIG. 3. Time taken by ova of *T. retortaeformis* to reach infective stage at different temperatures.

The fact that the eggs could also reach maturity at 3 to 5° was found accidentally while determining the temporary inhibitory effect of this temperature. A stock moist faecal culture of about 300 g (e.p.g. count, 1000) in a large glass jar was placed in a refrigerator at 3 to 5°. At successive weekly intervals about 10 g of faeces were removed and incubated at 25°. As the results were positive until the 9th week, it was decided to baermann the sample taken at the 10th week without further incubation. From this sample and from two similar samples 7 to 9% of infective larvae were recovered. Although the exact time taken by the ova to reach the infective stage at this temperature could not be determined accurately, it was estimated to be between 8 and 10 weeks.

The time interval in which the maximum number of eggs reach maturity at different temperatures was determined by keeping a number of uniform faecal cultures (each containing about 2700 eggs) at different temperatures and noting the number of infective larvae recovered at regular intervals. Table III records the results obtained.

TABLE III

Time taken by eggs to reach maturity at different temperatures

Temperature, ° C	Time taken
3 to 5	8 to 10 weeks
10 to 11	7 to 8 days
15	5 to 6 days
20	3 to 4 days
25	3 days
30	2 to 3 days
35	4 to 5* days
40	—

*Very small percentages reach maturity.

Effect of Low Temperatures

Faecal cultures containing about 1600–2500 eggs each were exposed to temperatures as low as −21°. The cultures were removed from the refrigerators at specified intervals and incubated at 25° for several days. The experiments were repeated several times.

It was found that eggs could resist continuous freezing at from −18° to −21° for 6 days, about 4 to 5% infective larvae being recovered after incubation. Eggs kept at 0° for 6 weeks yielded 11% infective larvae.

Effect of High Temperatures

Faecal cultures containing about 1800 eggs each, maintained at 100% R.H., were subjected to 40, 45, and 50° for specified periods. In order that the cultures might attain the desired temperature more quickly, water at 2° higher than the desired temperature was added to the Coplin jars before they were transferred to their respective incubators. Cultures were removed after specified periods, incubated at 25°, and the larvae recovered. Eggs remained viable for 18 hours at 40°, 1 hour at 45°, and 10 minutes at 50°.

Preinfective Stages

In the low temperature range preinfective stages were fairly resistant to freezing. In faecal cultures containing 1000 to 1500 preinfective larvae, about 15–20% resisted −18° to −21° for 6 days. When about 300 eggs were frozen in water at these temperatures for 8 days continuously, 33% revived after 12 hours at room temperature (23 to 25°). The same number of eggs when held in water at 0° showed a 70% survival after 10 days.

One to 1½-day-old cultures, containing about 2100 first-stage larvae which had been incubated at room temperature, when placed at 5° and 10° and examined after 16 days, were found to contain fully developed infective larvae. This suggests that once the eggs have hatched under normal conditions, their subsequent development is fairly normal, even under adverse conditions.

In the high temperature range, preinfective stages in faecal cultures survived 40° for 20 hours, but not for 24.

Infective Stages

Maximum Survival at Temperature Ranges of 0 to 40°

Infective larvae were isolated in tap water and about 500 placed in each of several Petri dishes in 3 ml of water. The Petri dishes were then placed in incubators at 5, 10, 15, 20, 25, 30, 35, and 40°. Fresh tap water was added from time to time to make up the loss caused by evaporation. The number of larvae surviving was made by actual count.

At the end of 5 to 6 weeks all the larvae at 0° were dead. At the end of the 13th week at 5 and 10° almost 90% were alive; at 15°, 74%; at 20°, 53%; at 25°, 37%; and at 30°, 19%. At 35° all the larvae were dead in 5 weeks, and at 40°, all were dead in 10 to 12 days.

These results indicate a greater tolerance of the larvae to low temperatures than to high ones. The larvae in the 35 to 40° range died sooner and were observed to cast their sheaths in the water just before they died, which may have made them more susceptible to bacterial action.

Longevity at Room Temperature

About 300 larvae in a little water were placed in each of three 50-ml beakers, covered by small Petri dishes, and left on a laboratory bench. The larvae were thus subjected to the effects of the day and night temperatures, etc., prevailing in the laboratory, and were thus under quite different conditions to those in incubators at constant temperatures and in total darkness. Regular biweekly studies were made of each culture, the number of larvae alive in each counted, and the mean recorded. Fresh tap water was added to restore the water level which had dropped during these intervals. Bacteria developed at the bottom of each vessel in due course and the larvae that died, decomposed and disappeared. The biweekly observations were continued until all the larvae had died. The results are shown in Table IV.

TABLE IV

Longevity of infective larvae at room temperature

Time, weeks	% survival	Time, weeks	% survival
2	99	12	83
4	98.5	14	66
6	96	16	40
8	95	18	6.5
10	88	19	0

As will be seen (Fig. 4), the longevity of these larvae at room temperature remained for a long time at a high level before reaching a sudden and rapid decline. The latter point was reached at the end of 12 to 14 weeks and all the larvae were dead by the 19th week.

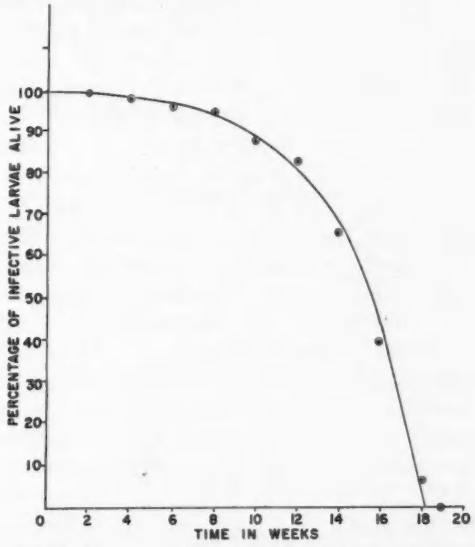

FIG. 4. Survival of infective larvae of *T. retortaeformis* at room temperature (20–26° C).

Effect of High and Low Temperature

In the high temperature range infective larvae survived for 2 to 3 days at 45°, but at 50 and 54° died in 2 and 1½ hours, respectively. For studies in a still higher temperature range, the larvae contained in a few drops of water in a centrifuge tube were dipped in water maintained at slightly higher than desired temperatures for specified periods. The results obtained after several trial experiments are recorded in Table V.

In the low temperature range, larvae were exposed to −21° for different periods and slow-thawed at 24°. After continuous exposure at this temperature for 8 days 5 to 7% of the larvae survived. They did not, however, survive for more than 24 to 36 hours after the initial low temperature exposure.

Fast freezing of less than half a minute lowered the temperature of the water containing larvae from +25° to −53°. After they were held at this temperature for 5 minutes, 4% of the larvae revived when slow-thawed at 25°. While most of the larvae had straightened or assumed the characteristic death posture, about 5% had burst at the anterior oesophageal region. Using the same technique but at a temperature of −60°, 2 to 3% of the larvae survived a 5-minute exposure, none a 10-minute exposure.

TABLE V

Viability of eggs at different relative humidities at room temperature (20°–26° C)

R.H., %	3 weeks		6 weeks		11 weeks		12 weeks		13 weeks	
	No.	%	No.	%	No.	%	No.	%	No.	%
20	525	26	81	4	39	2	10	0.5	0	0
44	485	24	69	3.5	0	0	0	0	0	0
66	201	10	29	1.5	0	0	0	0	0	0
81	130	6.5	19	1	0	0	0	0	0	0
92	110	5.5	7	0.35	0	0	0	0	0	0
100 (control)	Yielded 375 infective larvae and 445 preinfective larvae (dead or alive), that is, 19% and 22% respectively, after 5 days									

Relative Humidity

Relative humidity, an important determinant of the amount and rate of evaporation, is a critical climatic factor in the rate of moisture and temperature loss by plants and animals. Yet its importance as a major microenvironmental factor affecting the development and survival of free-living stages of nematodes has only recently been recognized.

Eggs of nematodes that undergo development on pastures before becoming infective to definitive or intermediate hosts, or before larvae hatch from them, require a certain amount of moisture. Larvae that hatch from these eggs cannot develop to the infective stage unless a certain amount of moisture is present. The degree of resistance to desiccation varies considerably both in the egg and the larvae.

While much has been written about the ability of these stages to resist desiccation, very few reports state their resistance in terms of actual degrees of dryness or percentage relative humidity. These figures usually denote the R.H. of the weather, not of the microclimate. In pastures, variations in R.H. occur even between the tip and base of blades of grass (1). Consequently, a clear picture of the adaptability of larval stages of nematodes to this vital microenvironment is rarely obtained. With this in mind, a series of investigations was undertaken.

Ova

Studies on the egg of *T. retortaeformis* have been limited to determining the developmental R.H. range at or near optimum temperatures and the viability of the egg at subdevelopmental ranges at room temperature.

Developmental R.H. Range

After it was found by preliminary experiments that 100% R.H. or 'actual wetness' was most suitable for the development of these eggs, it was decided to determine the minimum R.H. required by them to reach the infective or mature stage.

Faecal cultures containing 1200 to 2000 eggs were placed in each of a number of small Petri dishes with as little moisture as possible and incubated in a set of six air-tight wooden humidity chambers maintained at a R.H. ranging from 20 to 100% at room temperature (20 to 26°) for 1 week. The cultures were then removed and larvae collected by the Baermann technique. The results showed that the eggs are unable to complete their development at 92% R.H. and below. The minimum R.H. at which the eggs could hatch was somewhere between 92 and 100% R.H.

In order to determine the minimum R.H. range for development of the egg to the infective stage, tests were made at 93, 96.6, and 98% R.H. at 25°. Faecal cultures, each 5 to 10 g, containing about 2000 eggs, were placed in small wide-mouth, air-tight glass jars in which 50-ml beakers containing specific salt solutions could also be inserted. Faecal cultures in similar jars at 100% R.H. served as controls. All the jars were kept in a 25° chamber of the special microclimate cabinet. A fan in each chamber maintained a uniform temperature around each jar. At the end of the week cultures were removed and larvae collected by the Baermann technique. These experiments were repeated five times. No development took place at 93, and 96.6% R.H., a small one at 98% R.H., and a greater one at 100% R.H.

An estimate of the number of infective larvae from different cultures showed that though the larvae could complete their development at 98% R.H., only a small percentage (7–9%) actually did so. Even at 100% R.H. a culture failed to yield more than 25% ova as infective larvae. In contrast, cultures kept adequately moistened (actually wet) gave a yield of 70–85% under similar conditions.

It is evident, therefore, that these eggs have a very high moisture requirement for luxuriant growth—more than 100% R.H.—actual wetness is required.

Viability of Eggs at Subdevelopment R.H. at Room Temperature

The same wooden humidity cabinets, with a range of 20 to 100% R.H., were used. Room temperature was constant between 20 to 26°.

A set of five faecal cultures, each containing approximately 2000 eggs and with as little moisture as possible, were kept at 20, 44, 66, 81, and 92% R.H. Each was examined at intervals of 3, 6, 11, 12, and 13 weeks. The control culture was examined after 5 days only. Cultures were removed at the end of specified periods, placed in the Baermann apparatus for 12 to 15 hours, and 50 ml of bottom fluid collected from each. Eggs which had not hatched but were still viable usually hatched in the Baermann apparatus on coming in

contact with water. The number of these was either estimated by a dilution technique or counted under a binocular microscope. The results are shown in Table V.

It will be seen that these eggs have a remarkable capacity to resist desiccation; a small percentage (4 to 2%) were viable at 20% R.H. for up to 11 weeks and 0.5% for up to 12 weeks. There was a comparatively low recovery of freshly hatched larvae at higher R.H.'s at all examinations, and a complete absence of viable eggs as first-stage larvae from 44% to 92% R.H. from the 11th week onwards. The first may be due to the increasing numbers of eggs killed at higher R.H.'s and hence the comparatively low recovery, or, more probably, the eggs actually hatched prior to removal from the cabinets and the larvae had died and decomposed before examination. Only those eggs which had not hatched under these conditions were present as freshly hatched larvae in the Baermann apparatus.

The complete absence of viable eggs shown as freshly hatched larvae from the 11th week onwards in the 44 to 92% R.H. range can only be explained by assuming that these eggs have a somewhat wider hatching range of R.H. than the developmental range, and that within this range, hatching proceeds slowly until the end. On this basis, it can be seen that at 20% and 44% R.H. the effect is almost the same, with 66% R.H. intermediate between this and the higher R.H. Hence, it seems probable that the hatching limit of these eggs is approximately 55% R.H. Above this point there is a greater degree of hatching and fewer fatalities due to desiccation. Below this point, there is almost negligible hatching and greater fatalities due to desiccation. Within the range of 66% to 92% R.H. hatching probably continues until the 6th week, by which time all the possible eggs have hatched.

Infective Larvae

The resistance of *T. retortaeformis* larvae to drying at different percentages of R.H. was studied.

About 300 newly developed larvae were placed on layers of filter paper (Whatman No. 1) and each exposed to a R.H. of 20, 44, 66, 81, 92, and 100% at room temperature (20 to 26°). These were placed in the humidity cabinets and cultures removed at specified intervals. The larvae were transferred from the cultures by washing the cultures with water from the filter paper into the beaker. Trial experiments had shown that while the majority of larvae are recovered by this method, some remain and these were recovered by cutting the filter paper in two, three, or four small pieces and soaking them overnight in small Petri dishes containing about 5 ml of water, whence almost all the larvae passed into the water. This method not only made it possible to recover most of the living larvae but facilitated their immediate examination after they were transferred into the beaker. Immediate examination, under a binocular microscope, showed that in each case the larvae almost invariably assumed a tightly coiled position (similar to that of a watch spring), were

wrinkled, and retracted within their sheath. This may be due to a protective mechanism. Frequent and continued examinations showed that gradually (in about 10 to 12 hours) these larvae absorbed water, uncoiled, regained their normal shape, and became moderately active. In some cases, although the larvae regained their normal shape, they showed no activity and did not revive. The number of larvae revived in both the beaker and Petri dish were summed up and their percentage determined. The results are shown in Table VI.

TABLE VI

Percentage survival of larvae at various relative humidities at room temperature (20 to 26°)

R.H., %	Time, weeks						
	1	3	5	7	9	11	13
20	70	45	7	1	0	–	–
44	73	51	11	3	0	–	–
66	71	50	13	5	1	0	–
81	89	68	23	12	3	1	0
92	93	75	32	11	2	2	0
100	98	91	67	43	27	7	–

Table VI shows that these larvae, in general, resisted desiccation for a maximum period of 9 to 11 weeks. It also indicates that by the end of the 5th week most of the larvae at all relative humidities succumbed to the effects of desiccation and that only a small percentage survived for 9 to 11 weeks. In general, the mortality rate followed a more or less similar pattern, particularly in those at 20, 44, and 66% R.H. A somewhat similar resemblance could be seen at 81% and 92% R.H. The culture at 100% R.H., which also served as a control, showed a 27% survival at the end of the 9th week. Unfortunately, the culture dried and further readings after the 11th week had to be discontinued.

Conclusions

This study has shown that under carefully controlled laboratory conditions of temperature and humidity, that although the ova of *T. retortaeformis* will hatch and develop at temperatures ranging from 5 to 30°, the optimum temperature is 25°. Under conditions of actual wetness, these larvae reach the infective stage in from 3 to 4 days and can survive for over 75 days. Below this temperature hatching is progressively slowed until at 3 to 5° the infective stage is not reached for 8 to 10 weeks. Eggs will withstand actual freezing at −18 to −21° for 6 days but are quickly killed at temperatures over 40°. Mönnig (2) observed that in a cold, dry winter, development was slow and drying overtook *Trichostrongylus* eggs and larvae on pasture long before they reached the infective stage. He observed that preinfective larval stages were killed by freezing unless they were sheltered. He found that infective larvae were able to infect a lamb after having been frozen for 10 days. Crofton (1), in field experiments with *T. retortaeformis*, concluded that when the maximum temperature was below 12.8° there was no hatching. Zavadowsky (6)

recorded that *Trichostrongylus* spp. in Moscow did not resist desiccation. On the other hand, Mönnig (2) reported that eggs of *Trichostrongylus* spp. were resistant to drying in the embryonated stages and that he had recovered them after 15 months.

In this study, preinfective, free-feeding larvae, to the extent of about one-fifth of their number, survived −18 to −21° for 6 days in culture, and about one-third in plain water. Seventy per cent survived 0° for 10 days. While eggs developed best under conditions of actual wetness, some hatch at R.H.'s of 98% and above. They could, however, survive for long periods at low relative humidities (20 to 50% R.H.) at room temperature and below. While they survived higher R.H.'s, progressively fewer hatched when placed in wet cultures.

Infective larvae tolerate low temperatures better than high ones, 90% surviving at 5 to 10° for 13 weeks but none survived 40° for 10 to 12 days, and all died in a few hours at 50° and over. At room temperature, however, when conditions both of temperature and relative humidity varied, some larvae were still alive after 18 weeks, although there was a sudden decline after 12–14 weeks. Infective larvae could recover after freezing at −21° for over a week but lived for only a short time after thawing. Infective larvae will resist desiccation for about 11 weeks.

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ON THE TAXONOMY OF THE GENUS CLADOTAENIA, THE LIFE HISTORIES OF C. GLOBIFERA (BATSCH, 1786) AND C. CIRCI YAMAGUTI, 1935, AND A NOTE ON DISTINGUISHING BETWEEN THE PLEROCERCOIDS OF THE GENERA PARUTERINA AND CLADOTAENIA[1]

REINO S. FREEMAN

Abstract

A review of the taxonomy of the genus *Cladotaenia* established that at least four species of this genus occur in Europe, Egypt, and Sudan; that four species occur in North America; and that there are 10 valid species in the world. *C. circi* Yamaguti, 1935 is redescribed from *Circus cyaneus hudsonius* and *Accipiter cooperi*, and *C. globifera* (Batsch, 1786) is redescribed from *Accipiter gentilis atricapillus*, *A. striatus velox*, *Buteo p. platypterus*, *B. jamaicensis*, and *C. cyaneus hudsonius* all collected in North America. The development of the plerocercoid of both species is described. Natural infections with the plerocercoid of *C. circi* are reported from *Peromyscus maniculatus sonoriensis*, and with the plerocercoid of *C. globifera* in the rodents, *Clethrionomys gapperi*, *Napaeozapus insignis*, *Peromyscus maniculatus gracilis*, *P. leucopus noveboracensis*, *Tamias striatus*, *Tamiasciurus hudsonicus*, and the insectivore *Sorex cinereus*. The ratio of the length of the hook to length of guard of the large hooks of *Cladotaenia* is less than 3.5:1, whereas this ratio is more than 3.5:1 on the large rostellar hooks of *Paruterina*, which is considered a good criterion for distinguishing the plerocercoids of the two genera.

Introduction

Three species of metacestodes occur in the liver of certain small mammals at Algonquin Park, and elsewhere in Ontario. The life history of some of these cestodes has been studied over the past few years whenever material became available. An armed plerocercoid, different from the species reported previously (6), was relatively common, and apparently is the same species encountered several years ago at the University of Minnesota Biological Station at Itasca Park, Minnesota. For various reasons it was thought to be a species of *Cladotaenia* which matures in hawks. Therefore, adult worms were sought from hawks, and two species were recovered. Eggs from both species produced infections in mice which permitted study of the development of these worms. Studies on the morphology and life history of both species are reported here, together with an extensive review of the taxonomy of the genus *Cladotaenia*, and a note on how to differentiate between plerocercoids of this genus and those of the genus *Paruterina*.

Materials and Methods

Rodents and hawks from Ontario were examined as described previously (5, 6). Worms were recovered from five hawks, but they were immature in one and badly fragmented and decomposed in another bird, which precluded positive identification. The rostellum with hooks of some worms was removed,

[1]Manuscript received December 12, 1958.
Contribution from Department of Parasitology, Ontario Research Foundation, Toronto, Ontario, Canada.

flattened under cover glass, and studied in wet mount. Permanent preparations were made of such wet mounts by clearing in Beechwood creosote and mounting with Canada balsam. Eggs were studied in wet mount or in sectioned ripe proglottides stained with acid fuchsin or haematoxylin. Most ripe proglottides were removed to provide eggs for feeding studies. All worms were fixed in hot or cold 10% formol–saline, and some were stained with Mayer's carmalum or hemalum, or Ehrlich's acid haematoxylin.

Eggs were fed to laboratory mice either by stomach tube or mixed with moistened dog meal. Most plerocercoids were studied alive, and later whole mounts or sections were made by the same procedures used on the adult worms. Hawks ingested plerocercoids placed among the viscera of mice.

Young goshawks used for feeding studies were captured, while still nestlings, by Dr. G. F. Bennett. Wild adult male sharp-shinned hawks were kindly provided by Messrs. D. M. Wood and P. Mackenzie. Feces of all birds were examined, before and after experimental feeding, by routine sedimentation technique, but neither eggs nor proglottides were ever found.

Taxonomy

The genus *Cladotaenia* Cohn, 1901 was erected with *Taenia globifera* Batsch, 1786, from hawks, as the type. *T. dendritica* Goeze, 1782, from squirrels, was also included in the genus, but later it was removed and became the type of the genus *Catenotaenia* Janicki, 1904. *Cladotaenia* was included in the family Taeniidae Ludwig, 1886 by Cohn (3), where most workers place it today. Fuhrmann and Baer (9) moved the genus to the family Dilepididae Fuhrmann, 1907, subfamily Dilepidinae Fuhrmann, 1907 which will be followed here. Although the genus *Cladotaenia* was delineated (3), the proper name and morphology of the type species has remained confused. Some workers (8, 12, 15, 20, 34, and others) followed Fuhrmann (7) and considered *T. globifera* Batsch a synonym of *T. cylindracea* Bloch, 1782. However, as Cohn (3) and McIntosh (18) pointed out, *T. globifera* Batsch is single-pored and *cylindracea* is double-pored so they cannot be synonymous. Therefore, *T. globifera* must be the name of the type as originally designated. McIntosh (18) writes, however: "Neither Goeze's nor Batsch's descriptions and figures of this worm are adequate to enable it to be placed with certainty in any of the genera now recognized as valid. Morrell [sic] (1895) described a worm from *Buteo vulgaris* (= *Buteo buteo*) which he regarded as *T. globifera* Batsch. Since, as stated above, Batsch's description and figures are inadequate, it seems proper in order to avoid further confusion to accept Morrell's description of the worm from *Buteo vulgaris* (= *Buteo buteo*) as being that of the species named *T. globifera* by Batsch." Without doubt, the worm described by Morell (21) belongs in the genus *Cladotaenia*. The description lacks detail, however, as Cohn (3) indicated. Cohn (3) assumed that his specimens (recovered by Mühling in 1898 from *Circus rufus*) were the same species, and therefore Cohn did not describe his material completely.

Cohn showed, nevertheless, that his specimens differed from Morell's (21) in two significant ways. Firstly, Morell described a pouch-like oötype into which open the seminal receptacle and the ducts from the ovary, vitelline glands, and shell gland. Secondly, he described the testes divided into two lateral fields, which are not united posterior to the vitelline gland. Cohn found no such oötype and assumed Morell confused the developing uterus with the oötype. The testes in Cohn's specimens were united posterior to the vitelline gland creating a single U-shaped field. In addition the rostellar hooks on Cohn's specimens were smaller being 24 μ and 22 μ in two rows whereas Morell described only one hook 27 μ in length. It is possible that the two descriptions refer to two species of *Cladotaenia* with differences in hooks and testes distribution. This assumption is the basis for the discussion that follows.

This assumption is contrary to that of several workers, most recently Joyeux and Baer (12) and Schmelz (29), who have assumed that all *Cladotaenia* described from Europe constitute a single highly variable species. A critical review of the literature, even without outside supporting evidence which will be cited later, shows that the opposite is true. Then, which worm is *Taenia globifera* Batsch?

Von Linstow (14), who was the first to observe hooks on any *Cladotaenia*, and Mühling (22) figured and measured the hooks of worms they identified as *Taenia globifera* Batsch from *Buteo vulgaris*. The long and short hooks were identical in form and measured 34 μ and 26 μ, and 32.5 μ and 20 to 27.5 μ respectively. The accuracy of the minimum of 20 μ is open to question since Mühling states that some of the 46 hooks were drawn, and presumably measured, in different positions. Neither worker elaborated further on the morphology, rather referring the reader to other descriptions. Morell (21) recorded the hooks on his specimen as 27 μ long, but his drawing is sketchy. There is no mention of either the number of hooks, or whether they occurred in two rows. Since the large hooks usually fall from a scolex first, it is not unreasonable to assume that Morell measured the small hook, although this is not the interpretation made by Cohn (3), Schmelz (29), and others. Further, if a *Cladotaenia* hook is not in full side view it could present the view sketched by Morell. On the basis of the hooks, it is reasonably certain that the worms described by v. Linstow (14), Morell (21), and Mühling (22) as *Taenia globifera* Batsch from the same species of host (*Buteo vulgaris*) are the same species of worm. Thus *C. globifera* is described with testes in two lateral unconnected fields, with large hooks approximately 32 to 35 μ in length, and small hooks 27 μ or somewhat less in length. Limiting *C. globifera* to this concept leaves the worms described by Cohn (3) without a name, but as will be shown more fully later, his description fits *C. circi* Yamaguti, 1935.

Volz (33) redescribed, although incompletely, what he believed to be *T. globifera* with 46 hooks, the longer measuring 39.6 μ and the shorter 30.6 μ in length. He referred the reader to Morell's description for the internal anatomy of *T. globifera*. In the same paper Volz described a new species,

T. armigera, with 42 hooks, the two sizes measuring 39.6 μ and 32.4 μ respectively. Although *T. armigera* was described fully, only the hooks and a ripe proglottis were illustrated. The hooks of *T. armigera* are of the same shape as described for other species of the genus; therefore, it is unfortunate Volz did not illustrate hooks of the other species as well since he stated they are of different shapes. The greater lengths of hooks on both worms in contrast to what was described above for *C. globifera* suggest that none of Volz's material is this species. Even if one disregards the hooks, *C. armigera* (as described by Volz) can be distinguished from *C. globifera* (as described by v. Linstow, Morell, and Mühling) as follows: (i) the uterus is confined to the posterior third of proglottis and has four to seven uterine branches; (ii) the cirrus pouch measures 260 by 80 μ, which is appreciably larger than in any other species (12, 29); (iii) the scolex is stouter than on any other species described from Europe or North Africa.

Schmelz (29) followed Clerc (2) and attempted to establish that *C. globifera* is highly variable and that *C. armigera* is an extreme variant of this species. Schmelz maintained that the hooks of *C. globifera* (= *C. cylindracea* of Schmelz) are highly variable in length. As evidence he cited the following measurements from earlier literature: v. Linstow, 34 μ; Morell, 27 μ; Mühling, 32.5 μ; Volz, 39.6 μ; Cohn, 24 μ; Meggitt, 20–25 μ; Yamaguti, 20–35 μ. Actually, the measurements given by Yamaguti are taken from Lühe (15), who in turn cited v. Linstow and Mühling. Lühe listed the measurements of the large hooks from 32.5 to 35 μ in length and the short hooks from 20 to 27.5 μ in length, as was stated earlier. Schmelz did not mention, however, that Clerc examined a large number of specimens of *C. globifera* from three species of hawks and found the length of the hooks constant at 35 μ for the large and 25 μ for the small ones. As further support for his belief in the wide variation in hook size Schmelz stated: "... Joyeux et Baer (1936) qui ont contrôlé leurs mesures sur un matériel riche at varié, indiquent pour les grands crochets 28 μ à 39 μ et pour les petits 20 μ à 27 μ". Unfortunately the converse is true, Joyeux and Baer studied only limited material and the ranges they reported represent a partial summary of measurements reported by various authors (Baer, in litt.). Schmelz concluded therefore that the hooks of *C. globifera* range in length from 28 to 39 μ for the larger and from 20 to 27 μ for the smaller. Yet these ranges would still exclude the worms described by Cohn, who created the genus. However, if one does exclude the measurements given by Cohn, which really refer to another species as will be demonstrated below, as well as those given by Volz, for reasons stated above, then we see that the ranges in length for the large and small hooks of *C. globifera* are surprisingly constant at 32 to 35 μ and 27 μ or little less respectively, rather than being highly variable as maintained by Schmelz.

In regard to the uterus of *C. armigera* Volz, Schmelz (29) accepted the thesis presented by Clerc (2) that the uterus which Volz illustrated for *C. armigera* represented an extremely contracted proglottis resulting in a contracted uterus. Clerc maintained that he could find similar extremely

contracted uteri in a single strobila of *C. globifera* along with more normal uteri; he provided figures purporting to prove his point. Clerc's figures reveal the fallacy of his argument, however, since he showed the anterior margin of the uterus reaching to or beyond the level of the genital pore, quite unlike *C. armigera* in which the anterior margin of the uterus is far posterior to the level of the genital pore. Furthermore, a glance at Volz's illustration shows that the proglottis is not contracted. Thus the absolute length of the uterus is not as important as the position of the uterus within the proglottis.

There are two basic types of uteri in the genus *Cladotaenia*. In one group, which includes *C. globifera* and the majority of the species, the uterus extends from near the posterior margin to, or even considerably beyond, the level of the genital pore, thus extending two-thirds or more the length of the proglottis. In the second group, to which belong *C. armigera* and *C. foxi*, which will be discussed later, the uterus is confined to the posterior third of the proglottis, and the anterior end of the uterus is considerably posterior to the level of the genital pore. There may be an overlapping in the number of uterine pouches between *globifera* and *armigera*, depending partly on how one counts, but the latter typically has fewer than seven and the former has many more than seven pouches. There may be a third type of uterus, such as shown by *C. melierax* (Woodland, 1929), wherein the medullary parenchyma anterior to the uterus proper is so modified as to suggest a paruterine organ (2, 9, 16). More likely, however, this parenchymatous modification occurs to some extent in all species of this genus, but is overlooked because it is understained.

Schmelz (29) completely disregarded, and Clerc (2) cited erroneous figures for, the size of the cirrus pouch, which is considerably larger in *C. armigera* (260 by 80 μ) than the maximum (200 by 69 μ) given by Schmelz, or any other worker, for *C. globifera*. It is very significant that Fuhrmann (7) followed Clerc's action and synonymized *C. armigera* with *C. globifera*, but later Fuhrmann (8) examined the type of *C. armigera*, and concluded that it is a valid species.

Schmelz (29) recognized *C. freani* Ortlepp, 1938 as a valid species, and yet this species clearly falls into the more restricted concept of *C. globifera* given above. Likewise, *C. circi* Yamaguti, 1935 is considered valid by Schmelz, and yet Cohn's description of *C. globifera* differs in no significant way from Yamaguti's description of *C. circi*. Schmelz indicated in his table that *C. circi* occurs in Europe, but he did not elaborate how he arrived at this conclusion. Markowski (17) reported on experimental and natural infections of metacestodes of *Cladotaenia* in mice. He did not describe the adult worms, and identified all specimens as *C. cylindracea* (Bloch). However, Markowski's data indicate that he had specimens of *C. circi* and possibly *C. globifera*, as will be elaborated more fully below.

Fuhrmann and Baer (9) redescribed *Cladotaenia melierax* (Woodland, 1929) from the accipitriform bird *Astur badius sphenurus* from Africa. This species was originally placed in the genus *Rhabdometra*, and later removed to the genus

Paruterina by Fuhrmann (8), because Woodland (35) thought a thickening of the medullary parenchyma anterior to the uterus was a paruterine organ. Woodland's specimens lacked hooks. Fuhrmann and Baer (9) showed that this species lacks a true paruterine organ and has 38 hooks, the larger measuring 31 to 38 μ and the smaller 22 to 24 μ.

The above discussion leads to the conclusion that there are four species of *Cladotaenia* described from Europe, Egypt, and Sudan: (i) *C. globifera* Batsch (as in 14, 21, 22), (ii) *C. circi* Yamaguti, 1935 (see also 3), (iii) *C. armigera* Volz, 1900, and (iv) *C. melierax* (Woodland, 1929) (see 9). The worms described by Volz (33) as *C. globifera* must remain *species inquirendae*, since not even the host is known.

In 1900 Volz (33) summarized all records of cestodes taken from 20 species of hawks up to his time, and which were identified as *C. globifera* by various authors. Since, as shown above, at least four and possibly more species of *Cladotaenia* are involved, and since other genera occur in hawks as well, these records have little significance except in those cases enumerated above where the worms can be clearly identified. The short description of *C. globifera* (Batsch) which Joyeux and Baer (12) include in their monograph is therefore a composite of several species and this concept of this species has been followed by a number of subsequent workers (4, 23, 36).

There are a number of other descriptions and records of *Cladotaenia* from elsewhere in the world. As mentioned above *C. freani* Ortlepp, 1938 from South Africa is considered here a synonym of *C. globifera* and will be discussed further when this species is redescribed from North American specimens. *C. vulturi* Ortlepp, 1938 was described from four fragments, none fully ripe, which was recovered from a "vulture" in South Africa. There was one rostellar hook, 26 μ in length, and all measurements seem to accord closely with *C. freani* Ortlepp, 1938 except as Ortlepp (23) states: "...no testes are found lateral or posterior of the ovaries themselves...which is constant in all the segments and easily distinguishes this species from the preceding [*C. freani*] species." It would be desirable to examine further material to see if other characters such as the hooks and uterus would aid in separating this species.

C. fania Meggitt, 1933 from *Choriotis kori* (Charadriiformes) was described with 20 rostellar hooks from 6 to 7 μ in length, which readily distinguishes it from all other known species. It is the only species reported from a non-accipitriform bird, and, as the original description is incomplete, possibly it is not placed in the proper genus.

C. feuta Meggitt, 1933 from *Circus assimilis* was redescribed by Schmelz (29) from *Gypaetus barbatus* (Accipitriformes). This species is similar to *C. circi* differing primarily by having 92 to 96 rostellar hooks and 16 to 21 uterine pouches compared with 48 hooks and 7 to 10 pouches.

C. secunda Meggitt, 1928, described from an incomplete strobila, probably does not belong in the genus *Cladotaenia*. Meggitt (19) states his species is most nearly related to *Taenia lateralis* Fuhrmann, 1909 (now *T. hassalli* Fuhrmann, 1932), which is not a member of the genus *Cladotaenia*.

C. mirsoevi Skrjabin and Popov, 1924, from *Erinaceus transcaucasicus*, is the only record of this genus from an animal other than a bird. The name is a *nomen nudum*, however, and should be dropped.

The genus and species *Paracladotaenia accipitris* Yamaguti, 1935 was erected for a worm from *Accipiter virgatus gularis* collected in Formosa. *Paracladotaenia* has testes in two widely separated fields, a uterus which completely fills the ripe proglottis, and an internal seminal vesicle, but lacks hooks on the rostellum. As Schmelz (29) has already indicated it is most likely that the hooks had dropped from the prominent rostellum and that the other characters are not sufficiently different to erect a new genus; he therefore transferred the species to the genus *Cladotaenia*.

There are a number of records of *Cladotaenia* from North America including the description of three new species. These will be discussed following the descriptions of the material examined in the present study.

Cladotaenia Studied from North America

Sexually mature *Cladotaenia* were found in one marsh hawk, *Circus cyaneus hudsonius*. Sexually mature specimens of a second species of *Cladotaenia* were recovered from a juvenile goshawk, *Accipter gentilis atricapillus*, and a sharp-shinned hawk, *Accipter striatus velox*. One American rough-legged hawk, *Buteo lagopus sancti-johannis*, had *Cladotaenia* but their poor condition prevented specific identification. A single stained specimen of *Cladotaenia* from the broad-winged hawk, *Buteo platypterus platypterus*, was provided by the Ontario Department of Lands and Forests. In addition Dr. Glenn Hoffman, while at the University of North Dakota, kindly submitted five vials of adult *Cladotaenia*, four from marsh hawks and one "probably from a marsh hawk". Through the courtesy of Mr. A. McIntosh, specimens of *Cladotaenia* deposited in the U.S. National Museum also were available for study. Specimens referred to by Scott (31, 32) were made available by Mr. R. F. Honess.

The following hawks from Ontario either harbored no cestodes, or if cestodes were present, their condition precluded identification: one Cooper's hawk, three goshawks, seven plus one[2] sharp-shinned hawks, three red-tailed hawks, two broad-winged hawks, three plus one[2] marsh hawks, one sparrow hawk, *Falco sparverius*, one bald eagle. One osprey, *Pandion haliaetus carolinensis*, contained *Paradilepis rugovaginosus* Freeman, 1954.

The specimens from Ontario and North Dakota are recognized as two species, *C. circi* Yamaguti and *C. globifera* Batsch. In addition both species are present in the collection from the U.S. National Museum. Since neither species has been described from North American material, a detailed description incorporating all my material follows, with the hope that it will clarify the concepts of the two species. It is of interest that in Ontario *C. circi* was found in the field- and marsh-inhabiting marsh hawk and *C. globifera* in the

[2]Second number includes those birds harboring cestodes in such a condition that they were not identified.

forest-inhabiting sharp-shinned hawk, goshawk, and broad-winged hawk. On the other hand *C. globifera* occurred in the marsh hawk in North Dakota and red-tailed hawk in Oklahoma, whereas *C. circi* was taken from the forest-loving Cooper's hawk near Ithaca, New York.

Cladotaenia circi Yamaguti, 1935

Synonyms: *Cladotaenia globifera* Batsch (in Cohn (3))
C. cylindracea Bloch (in Meggitt (20))

Definitive hosts
in North America: *Circus cyaneus hudsonius* (marsh hawk)
Accipiter cooperi (Cooper's hawk)

Intermediate hosts
in North America: *Mus musculus* (experimental)
Peromyscus maniculatus gracilis (experimental)
P. m. sonoriensis

Locality: Canada and United States

Description

Dilepididae Fuhrmann, 1907; Dilepidinae Fuhrmann, 1907; *Cladotaenia* Cohn, 1901. Strobila 59 to 82 mm in length,[3] greatest width 1.4 to 1.6 mm usually near region of sexual maturity.[4] Anterior part of strobila somewhat serrate, terminal part more moniliform; 232 to 259 proglottides. Immature proglottides much broader than long; mature segments from 0.41 mm in length by 1.38 mm in width to 0.62 by 1.11 mm; ripe segments more variable depending on state of contraction 1.41 by 0.98 mm to 3.80 by 0.81 mm. Region of sexual maturity begins at proglottis 190 to 225. Scolex variable in shape from 173 μ in length by 214 μ in width at suckers to 214 by 231 μ; four suckers from 76 by 76 μ to 103 by 97 μ; rostellar pad from 35 by 74 μ to 56 by 90 μ. With 54 to 60 rostellar hooks;[5] large hooks from 22 to 24 μ in length, and small hooks from 18 to 20 μ in length (Fig. 1). Ratio of total length to length of guard (*ab* : *cd*, Fig. 1) of large rostellar hooks 2.4 to 2.9: 1 av. 2.65: 1, and for small rostellar hooks 2.6 to 3.0: 1 av. 2.9: 1. Neck from 470 to 680 μ in length by 58 to 104 μ in width at narrowest point. Genital atrium irregularly alternate, one-third to one-fifth way from anterior margin, highly variable in shape from shallow saucer shape to narrow cleft depending on position of cirrus. Testes 62 to 89 in number, ovoid from 49 by 18 μ to 72 by 58 μ, confined to two lateral fields not overlapping excretory ducts laterally and not crowding into area anterior to ovary, usually more testes in aporal field, both fields extending full length of proglottis, and two fields united by one or more rows of testes along the posterior margin of the proglottis behind the female genital complex (Fig. 2), in other specimens not so united. Vas deferens, with highly convoluted portion which extends from

[3]The range determined from 11 slides of whole mounts.
[4]Region of sexual maturity begins where definite lumen occurs in developing uterine stem.
[5]Determined from the plerocercoid.

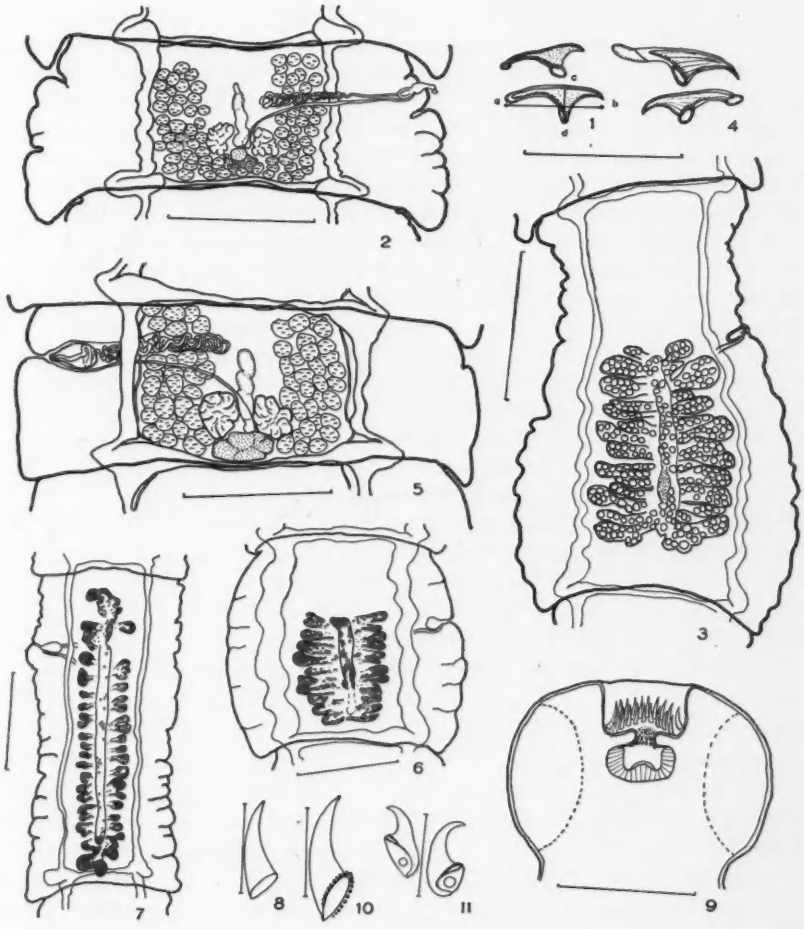


FIG. 1. Large and small rostellar hooks of *Cladotaenia circi* from plerocercoid from experimental *Mus musculus*. FIG. 2. Mature proglottis of *C. circi* from marsh hawk. FIG. 3. Ripe proglottis of *C. circi* from marsh hawk. FIG. 4. Large and small rostellar hooks of *C. globifera* from sharp-shinned hawk. FIG. 5. Mature proglottis of *C. globifera* from goshawk. FIG. 6. Slightly contracted ripe proglottis of *C. globifera* from goshawk. FIG. 7. Partially stretched ripe proglottis of *C. globifera* from sharp-shinned hawk. FIG. 8. Freehand drawing of conical blade of developing rostellar hook of *C. globifera*. FIG. 9. Drawing made from photomicrograph of optical section of developing scolex on plerocercoid of *C. globifera*, showing relationship of conical blades, hyaline particles, and rostellar pad within scolex canal. FIG. 10. Freehand drawing of more advanced blade with granules which form guard bulb of rostellar hook of *C. globifera*. FIG. 11. Drawing from photomicrograph showing more advanced rostellar hooks of *C. globifera*; note remnants of cell and nucleus within which hook was developing.

All drawings done with the aid of a camera lucida unless otherwise indicated. Scales on FIGS. 8 and 10 = 10 μ; FIG. 11 = 20 μ; FIGS. 1 and 4 = 40 μ; FIG. 9 = 50 μ; FIGS. 2, 3, and 5 = 0.5 mm; and FIGS. 6 and 7 = 1.0 mm.

near middle of proglottis to position lateral to ventral excretory duct, opening directly into cirrus pouch. No internal or external seminal vesicles. Proximal margin of cirrus pouch always far lateral to poral edge of ventral excretory duct, usually with cirrus everted. Cirrus pouch from 55 by 28 μ to 97 by 53 μ when cirrus everted, but 83 by 65 μ to 118 by 61 μ when cirrus retracted. Extended cirrus unarmed, thick at base narrowing toward distal end, approximately 90 μ in length by 35 μ in width at base. In immature proglottides genital ducts pass between dorsal and ventral longitudinal excretory ducts, but in more mature proglottides the dorsal ducts frequently difficult to see. Excretory ducts highly variable in dimensions, but ventral and transverse duct on posterior margin of proglottis visible throughout length of strobila (Figs. 2, 3). The ventral excretory duct is sharply constricted at the posterior margin of each proglottis at a position anterior to where transverse commissure begins. Vagina about 500 μ in length and 10 to 20 μ in width, extending mediad from opening posterior to cirrus at base of genital atrium until in the vicinity of the poral wing of the ovary the vagina arches caudad and proceeds to near the isthmus of the ovary and opens into the seminal receptacle lying ventral to the isthmus between the wings of the ovary. Wings of ovary subequal and lobate; total size of ovary from 56 by 168 μ to 93 by 347 μ. Vitelline gland, transversely elongate, frequently granular, from 69 by 70 μ to 49 by 139 μ. Mehlis' gland, approximately 46 μ in length by 58 μ in width lying in hollow in anterior face of vitelline gland. Uterus begins development ventral to ovarian isthmus and extends anteriorly along mid-line of proglottis. In fully ripe proglottides uterus reaches slightly anterior to level of the genital atrium, with 8 to 14 lateral pouches on each side (Fig. 3); some of the ripest proglottides with medullary parenchyma anterior to uterus forming pouch-like structure. Unfixed egg (Fig. 12) measuring approximately 33 by 26 μ with embryo 24 by 25 μ. The egg shell thin with pronounced globular (granular?) substance between shell and embryo in unfixed eggs; buttons lacking. Three pairs of similar embryonic hooks approximately 8 to 9 μ in length. Fixed eggs from 24 by 26 μ and 22 by 31 μ to 28 by 30 μ.

Cladotaenia globifera (Batsch, 1786)

Synonyms: Taenia globifera Batsch (in v. Linstow (14); Morell (21); Mühling (22))

Cladotaenia freani Ortlepp, 1938

Cladotaenia oklahomensis Schmidt, 1940

Definitive hosts

in North America: *Accipiter gentilis atricapillus* (goshawk)

A. striatus velox (sharp-shinned hawk)

Buteo p. platypterus (broad-winged hawk)

B. jamaicensis (red-tailed hawk)

Circus cyaneus hudsonius (marsh hawk)

Intermediate hosts
in North America: Rodentia
Mus musculus (experimental)
Clethrionomys gapperi
Napaeozapus insignis
Peromyscus maniculatus gracilis
Tamias striatus
Tamiasciurus hudsonicus
Insectivora
Sorex cinereus
Locality: Canada and United States

Description

Dilepididae Fuhrmann, 1907; Dilepidinae Fuhrmann, 1907; *Cladotaenia* Cohn, 1901. Strobila about 120 to 348 mm in length,[6] greatest width from 1.76 to 2.96 mm, near region of sexual maturity. Proglottides variable in shape depending on degree of contraction, terminal part of strobila may be moniliform; 211 to 482 proglottides. Immature segments much broader than long; mature segments usually broader than long, from 0.95 by 0.68 mm to 1.12 by 1.12 mm to 0.92 by 1.90 mm; ripe segments from 2.00 by 2.36 mm and 2.75 by 1.54 mm to 4.28 by 1.06 mm. Region of sexual maturity begins at proglottis number 170 to 270. Scolex from 162 μ in length by 346 μ in width at suckers to 231 by 306 μ; four suckers varying from circular to ovate, 97 to 125 μ in maximum dimensions; rostellar pad from 46 by 72 μ to 97 μ wide, rostellar ring 120 μ in diameter. From 44 to 54 rostellar hooks, arranged in two rows; large hooks 31 to 34 μ, small hooks 21 to 26 μ (Fig. 4). Ratio of total length to length of guard of large hooks 2.9 to 3.3: 1 av. 3.2: 1, and for small rostellar hooks 3.0 to 3.7: 1 av. 3.4: 1. Dimensions of neck difficult to measure, 202 to 404 μ in length by 230 to 375 μ at its narrowest point. Genital atrium irregularly alternate about one-third to one-fourth way back from anterior edge of proglottis, variable in shape, but usually narrow and deep. Testes from 60 to 81, usually ovoid 67 by 53 μ to 86 by 72 μ; confined to two lateral fields approximately uniform in width throughout, extending the length of proglottis, not overlapping excretory vessels laterally, and fields not uniting posterior to female genital complex in some (Fig. 5), but united in other specimens. Vas deferens convoluted, extending from middle of proglottis to cirrus pouch; no internal or external seminal vesicles. Proximal margin of cirrus pouch reaches near but seldom overlaps poral margin of ventral excretory vessel. Cirrus pouch pyriform from 81 by 62 μ to 97 by 83 μ in specimens from sharp-shinned hawk, 114 by 83 μ to 139 by 76 μ in marsh hawk, and 146 by 90 μ to 187 by 105 μ in goshawk and red-tailed hawk; cirrus usually not everted. Genital ducts pass between dorsal and ventral longitudinal excretory ducts. Dorsal excretory duct fairly uniform and usually visible throughout length of strobila; ventral and transverse ducts

[6]Measurements from 14 worms in whole mount.

more variable. Ventral duct sharply constricted at posterior margin of each proglottis anterior to where transverse commissure begins (Fig. 5). Vagina about 725 μ in length by 15 to 17 μ in width opening into genital atrium posterior to cirrus and extending mediad to seminal receptacle ventral to ovarian isthmus. Ovary with subequal lobulate wings united by isthmus; total size of ovary from 100 by 229 μ to 243 by 347 μ. Vitelline gland lobulate, from 62 by 160 μ to 104 by 260 μ, transversely elongate with concave anterior face in which sits Mehlis' gland measuring from 69 by 90 μ to 72 by 97 μ. Uterus begins development in vicinity of the ventral side of the ovarian isthmus and extends anteriorly along the mid-line of the proglottis. In fully ripe proglottides uterus may extend from near posterior margin of proglottis to just anterior to level of genital pore in some proglottides and almost the entire length of other proglottides (Figs. 6, 7); with 9 to 24 lateral pouches on each side of uterine stem, exact number usually difficult to count; in some fully ripe proglottides medullary parenchyma anterior to uterus forming pouch-like structure. Unfixed egg (Fig. 13) measuring about 42 by 32 μ in over-all dimensions; embryo varying from 28 by 21 μ to 30 by 23 μ. Egg shell non-striated and 2 to 4 μ thick. Egg with a button-like structure at each end of embryo between the embryo and the egg shell, usually measuring approximately 10 by 4 μ but varying from 8 by 6 μ to 12 by 4 μ (Fig. 13). Embryonic hooks almost alike, 8 to 9 μ in length; blade more sharply curved and guard slightly more pronounced on lateral hooks than on median pair of hooks. Fixed eggs measuring from 21 by 18 μ to 31 by 28 μ.

Representative whole mount preparations of adults have been deposited in the U.S. National Museum as follows: C. *circi* from marsh hawk No. 56256; *C. globifera* from goshawk No. 56257, sharp-shinned hawk No. 56258, and marsh hawk No. 56259, No. 56260, and No. 56261. The following slides of metacestodes also were deposited: *C. circi* from *Mus musculus* No. 56247, and from *Peromyscus maniculatus* No. 56248; *C. globifera* from *M. musculus* No. 56249 and No. 56250, from *P. maniculatus* No. 56251 and No. 56252, from *Sorex cinereus* No. 56253, from *Tamias striatus* No. 56254, and from *Clethrionomys gapperi* No. 56255.

Discussion of the Species of *Cladotaenia* in North America

C. circi from Ontario is appreciably smaller than *C. globifera*, has smaller rostellar hooks with a ratio of total length to length of guard of large rostellar hooks less than 3.0:1, and has the testicular fields united posterior to the female genital complex. *C. globifera* from Ontario has the testes in two lateral fields usually not united posterior to the female genital complex, ratio of total length to length of guard of large rostellar hooks usually more than 3.0:1, and has a button on each end of the egg between the embryo and egg shell, whereas *C. circi* lacks such buttons. All complete specimens from North Dakota are as large as or larger than any of the specimens from Ontario, but the testicular fields are united by a posterior strand of testes in most

proglottides. Nevertheless, the size and proportions of the rostellar hooks and the large size of the strobila mark them as *C. globifera*. One scolex fragment from one marsh hawk in North Dakota had three small rostellar hooks all 21 μ in length, but the ratio of total length to length of guard ranged from 3.2 to 3.5:1, which indicates it is *C. globifera*. The morphology of *C. circi* from a Cooper's hawk (U.S.N.M. No. 47092) collected at Ithaca, New York, agreed in every respect with the specimens from Ontario except that the testes fields usually were not united. Thus, it is apparent that the testicular fields may be united or separate in both species and that this is not a reliable character for separating these two species.

The size and general morphology given in the original description of *C. oklahomensis* Schmidt, 1940 from Oklahoma closely agree with the above description of *C. globifera*. In addition Schmidt (30) described the hook lengths as 28.8 μ for the large and 19.4 μ for the small hooks, but his camera lucida drawings disagree with his description. Re-examination of a type slide (U.S.N.M. No. 36699) showed that the large hooks are at least 31 to 32 μ in length and the small ones about 24 μ. Also this specimen had 73 to 80 testes, whereas Schmidt reported 93 to 104. Even the uterus with numerous short alveoliform pouches, as described by Schmidt, is closely simulated by some specimens of *C. globifera* from Ontario (Fig. 7); therefore *C. oklahomensis* Schmidt is placed in synonymy with *C. globifera* Batsch.

Both *C. freani* Ortlepp, 1938, from the black eagle hawk (? *Aquila* (= *Pteroaetus*) *verreauxi* (Less.)) in South Africa, and *C. globifera* Batsch as described herein agree with the generalized description of *C. globifera* from Europe discussed earlier. Ortlepp (23) stated in regard to *C. freani* that, "*Cladotaenia cylindracea* is very similar to the writer's species in that testes do not encroach into the ovarian field anterior to the ovary, but differs from it in that its testes pass posteriorly and meet behind the yolk glands, and because the median stem of the uterus in the writer's species may reach the anterior border of the segment." The concept of *C. globifera* (=*C. cylindracea*) which Ortlepp used was as presented by Joyeux and Baer (12). As shown above all these characters are common to *C. globifera*; therefore, *C. freani* is reduced to synonymy with it. There are minor differences between the description given by Ortlepp (23) and that given here, namely: (1) the strobilae from Africa are smaller, but they are "considerably shrunken" (23), (2) there are 80 to 100 testes compared with 60 to 81 in the specimens from Ontario, (3) the testes in the latter are larger, and (4) the cirrus pouch, although the same size in both, is far removed from the ventral excretory vessels in the African specimens but may overlap this duct in the North American specimens. The number of uterine pouches varies from 10 to 24 in our specimens, but generally the number is from 15 to 19; Ortlepp (23) found from 10 to 14.

The specimens described here as *C. circi* are, with minor variations, like those described under the same name by Yamaguti (36), and like *C. globifera* of Cohn (3), and *C. cylindracea* of Meggitt (20). The large and small hooks

are surprisingly constant in size among them all, but the number varies from 46 (Cohn), 48 (Yamaguti), 56 (Meggitt) to 54 to 60 av. 57 (this study). Likewise the testes distribution is similar in all four descriptions, but the number present varies from 64 to 67 (Meggitt), 64 to 89 (this study), 90 to 110 (Yamaguti), not reported (Cohn). The cirrus pouch is variable also, being 55 by 28 μ to 97 by 53 μ when the cirrus is everted but up to 118 by 61 μ with cirrus retracted (this study), 120 μ long (Cohn), 120 to 150 by 50 μ (Yamaguti), and 140 to 150 by 59 to 69 μ (Meggitt). In Meggitt's specimens the cirrus sac extends to the excretory ducts, but in all the other descriptions, especially the present one, it does not extend that far. The number of uterine pouches varies from 7 to 10 (Yamaguti), 11 to 14 (Meggitt), 12 to 14 (this study), and "numerous" (Cohn).

Mounted specimens from a marsh hawk (U.S.N.M. No. 28048) collected from near Washington, D.C., and from the short-eared owl, *Asio flammeus* (U.S.N.M. No. 28058) collected at Miles City, Montana, reported by Jones (11) as *C. globifera* were examined. The cestodes from the marsh hawk lacked hooks, were fragmented, and otherwise were difficult to study, but appeared to be *C. circi*. The specimen from the owl was about 60 mm long and had approximately 50 rostellar hooks of the size and shape of *C. circi*. The cirrus pouch was up to 194 by 83 μ in size, however, and overlapped the ventral excretory vessel, which is much larger than on any other *C. circi* and slightly larger than any *C. globifera* which were examined. There were approximately 60 testes in two fields united posteriorly. The uterus was diffuse and lacked distinctive pouches, but this may be due to poor fixation. There was a very pronounced thickening of the medullary parenchyma anterior to the uterus forming a pseudoparuterine organ as reported by Clerc (2), Fuhrmann and Baer (9), and Mahon (16). This is the only record of this genus from an owl, and possibly it is a new species. Better specimens are required, however, before a complete description is possible.

The collection from the U.S. National Museum included a slide of a *Cladotaenia* (No. 41678) from a "Falcon" collected at Igloolik Island, Northwest Territory, Canada. As it lacked a scolex and the oldest proglottides were not fully mature, it was not identified to species.

A type specimen of *C. banghami* Crozier, 1946, from *Haliaeetus leucocephalus* (U.S.N.M. No. 36940), and one of *C. foxi* McIntosh, 1940, from *Falco peregrinus anatum* (U.S.N.M. No. 44445), the only other species of this genus described from North America, also were examined. *C. banghami* consisted of several slides of strobilar fragments and a squashed scolex of an incomplete worm. There were 34 hooks, the large ones measuring 37 to 39 μ, the small ones 29 μ. The testes were in two fields united in some proglottides and distinctly separate in others, with 100 to 120 testes per proglottis, which is more than in any cestode of this genus examined from North America. The uterus was not distinctly visible in the ripe proglottides. The smaller number of hooks and their large size serve to distinguish this species from others in the genus.

C. foxi is distinctive, being a stout worm with a huge scolex, widely separated testicular fields, and a uterus confined to the posterior third of the proglottis. It is unlike any other member of the genus from North America, and resembles *C. armigera* except that the latter has much larger rostellar hooks, united testicular fields, and a cirrus pouch which is twice as large. Preble (26) recovered six *C. foxi* from a duck hawk in Ohio.

Scott (31, 32) stated that he had found two new species of *Cladotaenia* from North American hawks, but the descriptions remain unpublished. The author examined all of Scott's specimens, through the courtesy of Mr. R. F. Honess of The University of Wyoming, and found that two of the three ferruginous rough-legged hawks which Scott fed experimentally later harbored *C. globifera*, and one had a mixed infection with *C. globifera* and *C. circi*.

Penner (24) gave a preliminary report on life history studies with worms he later (25) identified as *C. circi*.

Key to Species of *Cladotaenia*[7]

1. Uterus short, anterior end about half way from posterior end of proglottis to level of genital pore 2
   Uterus long, anterior end reaching near or anterior to level of genital pore 3
2. Large and small hooks about 30 and 28 μ respectively ..... *C. foxi*
   Large and small hooks about 40 and 32 μ respectively ..... *C. armigera*
3. Posterior margin of testis fields does not reach posteriorly to level lateral of ovary. *C. vulturi*
   Posterior margin of testis fields reaches posteriorly at least to posterior margin of ovary, and fields may unite posterior to female reproductive organs 4
4. Uterus fills entire space between ventral excretory vessels and transverse excretory ducts ..... *C. accipitris*
   Uterus more restricted than above 5
5. Large rostellar hooks 10 μ or less in length ..... *C. fania*
   Large rostellar hooks over 20 μ in length 6
6. About 90 rostellar hooks ..... *C. feuta*
   Less than 70 rostellar hooks 7
7. Under 40 rostellar hooks; large hooks usually 35 μ or more in length 8
   Over 40 rostellar hooks; large hooks usually under 35 μ in length 9
8. Uterus with five to seven lateral pouches, and prominent pseudoparuterine organ anterior to uterus ..... *C. melierax*
   Uterus with 11 to 19 lateral pouches, pseudoparuterine organ lacking or not prominent ..... *C. banghami*
9. All rostellar hooks 25 μ or less in length ..... *C. circi*
   Large rostellar hooks 28 μ or more in length ..... *C. globifera*

The Life History of *Cladotaenia globifera* (Batsch)

Eggs of *Cladotaenia globifera* (Fig. 13), which were obtained from ripe proglottides collected from an immature sharp-shinned hawk, infected a series of nine *Mus musculus* and one of two *Peromyscus maniculatus gracilis*. The mice were killed at irregular intervals beginning 10 days later in order to study the development and morphology of the plerocercoid.

Two mice, killed 10 days post-feeding, had extensive diffuse lesions near the periphery of the lobes of the liver as well as some scattered throughout it. The mesenteric lymph node of one mouse was swollen, although neither animal

[7] *C. mirsoevi*, which is *nomen nudum*, is omitted from key.

had metacestodes in this organ. Intact lesions were removed from the liver for study, but nearly half had no associated metacestodes, and frequently when metacestodes were found they were to one side of the lesions. Each of the living metacestodes had a gelatinous layer intimately surrounding all or most of it (Figs. 14, 16). Several lesions had dead and disintegrating metacestodes.

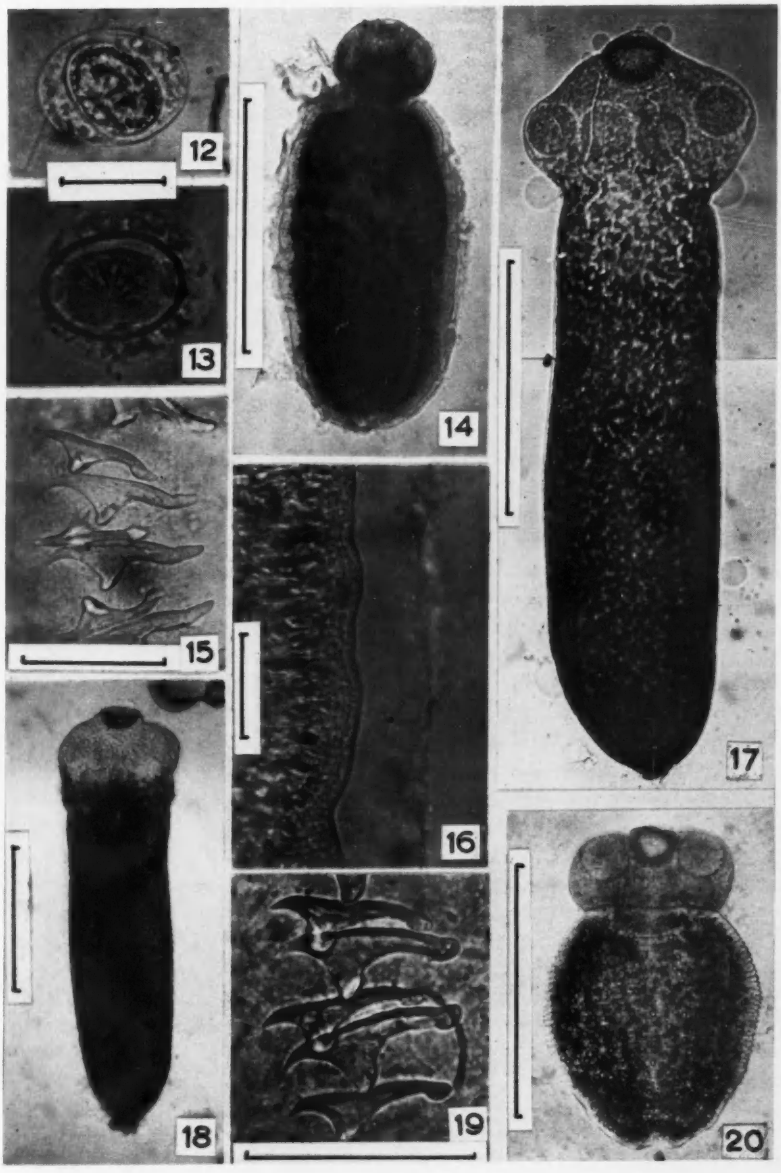
None of the metacestodes was fully developed, and they ranged from forms with the earliest trace of sucker and rostellar development to others with advanced sucker development and developing rostellar hooks. None had a lacuna in the parenchyma. The plerocercoids ranged up to 1.41 by 0.41 mm in size when flattened under cover glass, but most specimens were less than 0.80 by 0.30 mm when not flattened.

The plerocercoid which was least developed measured approximately 1.20 by 0.40 mm and on it the sucker and rostellar primordia could just be detected, but there was no evidence of hook development. The scolex was slightly more advanced on several other specimens and had a more clearly delineated rostellar pad and suckers, although the latter only suggested the cup-like formation which develops later. The incipient rostellar hooks consisted of many particles of hyaline material varying in size and shape and apparently scattered on the wall of the scolex canal anterior to the developing rostellar pad; there were many more of these particles than the resulting number of hooks, and in some plerocercoids they appeared to be in four irregular rows. Some calcareous corpuscles were evident throughout the body.

In more advanced specimens the blade of the hook was well outlined, although thin (Figs. 8, 9), but there was no trace of guard or handle formation, and the blade was open on the handle end. Many of the particles of hyaline material mentioned earlier were still present confined posterior to the developing blades on the wall of the scolex canal (Fig. 9). On still more advanced plerocercoids a ring of small granules, forming the primordium of the guard bulb, encircled the base of the blade (Fig. 10), and by this time most of the hyaline particles were gone. In plerocercoids such as these the suckers were more advanced, but still not fully muscular and cup-like. Calcareous corpuscles were now prominent as were many parts of the excretory system.

FIG. 12. Egg of *C. circi* from marsh hawk; note yolk envelope surrounding egg proper, and presence of granules, but the lack of buttons between egg shell and embryo. FIG. 13. Egg of *C. globifera* from sharp-shinned hawk; yolk envelope out of focus, but note prominent buttons on each end of embryo between it and egg shell. FIG. 14. Entire 10-day-old plerocercoid of *C. globifera* from experimental *M. musculus*; note gelatinous layer surrounding most of plerocercoid. FIG. 15. Rostellar hooks of *C. globifera* from plerocercoid from liver of *Clethrionomys gapperi* collected in Minnesota. FIG. 16. Part of body wall and gelatinous layer under higher magnification from plerocercoid in FIG. 14. FIG. 17. Fully developed plerocercoid of *C. globifera* 343 days old from experimental *M. musculus*. FIG. 18. Fully developed plerocercoid of *C. circi* 14 days old from experimental *M. musculus*. FIG. 19. Lateral view of rostellar hooks of *C. circi* from 14-day-old plerocercoid from experimental *M. musculus*. FIG. 20. Nearly fully developed 14-day-old plerocercoid of *C. globifera* from experimental *M. musculus*; note superficial bladder-like appearance.

FIGS. 12 through 20, photomicrographs. Scales on FIGS. 12, 13, 15, 18, and 20 = 30 μ, and on FIGS. 14, 16, 17, and 19 = 0.5 mm.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The excretory atrium was formed, but readily prolapsed with slight pressure of the cover glass. The embryonic hooks were embedded lateral and anterior to the atrium in the position they occupy in fully developed plerocercoids. The small dorsal and larger ventral excretory ducts, along with many flame cells, were noticeable along the lateral margins of the plerocercoid. There were auxiliary ducts branching from the dorsal duct, particularly toward the posterior end of the plerocercoid. Direct connections between the flame cells and the excretory ducts were not observed. The two lateral dorsal ducts turned mediad and united anterior to the excretory atrium, but the ventral ducts became transparent in the vicinity of the atrium and may have been discontinuous. The most advanced plerocercoid in the 10-day-old infection measured 1.41 by 0.41 mm when slightly flattened under cover glass. The scolex was evaginated and the hooks fell off facilitating study in full side view. These hooks apparently grew within a single cell, and parts of the parent cell were still evident (Fig. 11). The handle was now partially developed, but lacked any trace of the handle bulb, whereas the guard bulb was more massive than before, albeit still not fully formed. The suckers and excretory system were as described for the previous specimen.

Two mice killed 14 days post-feeding still had diffuse lesions as well as more clearly defined spots on the liver. The latter lesions either contained traces of overcome plerocercoids or debris which was not identified; living plerocercoids were associated with the more diffuse lesions. All plerocercoids, although showing variation in development among themselves, were more advanced than from 10-day-old infections. The development of the rostellar hooks was most variable, the large hooks varying from 25 to 32 μ in length and the small ones from 21 to 26 μ in length. The least-developed hooks were open at the end of the developing handle and lacked a handle bulb, whereas the handles of the advanced ones were sealed by a small handle bulb. The gross morphology of the armed plerocercoids was now distinct and they were smaller than the maximum size of plerocercoids which were 10 days old. One plerocercoid measured 0.635 mm in length by 0.440 mm in width, the scolex 160 μ in length by 330 μ in width, the suckers 93 μ, and the rostellar ring 83 μ, with approximately 40 hooks. The shape of a contracted plerocercoid superficially resembles a cysticercus (Fig. 20), which may explain why some workers (13, 17, 18) have called similar plerocercoids cysticerci.

By 21 to 22 days post-feeding the liver contained only small discrete cysts, which were either opaque or clear-walled, and occurred primarily along the periphery of the lobes. Apparently a balance between the host and parasite was now established, since the cysts did not change appreciably with further passage of time except to become more hardened. Even 575 days after the animals were fed, many cysts remained clear-walled and plerocercoids were observed within them. Frequently fully formed cysts were difficult to open due to the thick slippery wall. Some plerocercoids were immobile when first removed, although most became active and usually evaginated when placed in saline, if the scolex had not evaginated already while it was encysted.

Most plerocercoids appeared fully developed within 3 weeks, although rostellar hooks on some 22-day-old plerocercoids still lacked a fully formed bulb on the handle or guard and such large hooks measured only 29 μ in length. The hooks on some plerocercoids may remain permanently stunted, however, for on some from a 343-day-old infection the large hooks measured only 27 μ in length, although otherwise normal except for a short almost spherical bulb on the handle.

The following description of the fully developed plerocercoid of *C. globifera* (Fig. 17) is based on measurements from 16 living or fixed specimens from five infected *Mus musculus* and one *Peromyscus maniculatus gracilis* killed 21 to 343 days after infection. There were no significant differences among specimens from the two hosts. The range of measurements of the fixed specimens was similar to the living ones, therefore the data are combined. The length and width of plerocercoids with everted scoleces varied from 0.635 by 0.320 mm to 0.800 by 0.335 mm to 0.960 by 0.225 mm, whereas one specimen with an invaginated scolex measured only 0.480 by 0.180 mm. The scolex varied from 225 to 335 μ in width and from 150 to 200 μ in length depending on the extent of evagination; if invaginated the scolex cone was approximately 200 μ in length. The suckers varied from 69 by 49 μ to 104 by 67 μ with the maximum dimension usually between 80 and 90 μ. The rostellar ring varied from 46 to 105 μ across depending on the degree to which the hooks were spread; the rostellar pad likewise varied from 42 by 67 μ to 56 by 90 μ. There were 44 to 54 rostellar hooks (av. 50); the large hooks varied from 26 to 34 μ (av. 31 μ) in length and the small ones from 22 to 26 μ (av. 24 μ) in length (Fig. 15). Generally the hooks from the lower end of the range had a stunted bulb on the handle.

The excretory system, studied on living specimens and stained whole mounts and sections, has the basic pattern described for the armed plerocercoids of *Paruterina* (6). There were some differences, however, and certain features, particularly some associated with the dorsal duct, stood out more clearly on the present material, so a detailed description is included here.

The small dorsal and the larger ventral excretory duct were visible along both lateral margins. In the scolex the four ducts were interconnected by commissures so as to form a rough rectangle when the scolex was viewed *en face* with one of the lateral ducts entering the rectangle at each corner. The arrangement of the ducts was more complicated toward the posterior end of the plerocercoid than observed in *Paruterina*. The dorsal ducts, which did not extend as far posteriorly as the ventral ducts, bent mediad and united to form a continuous, albeit not simple, tubule. The duct showed as many as four bifurcations, although the branches of each bifurcation soon reunited creating the appearance of an opening in the duct. In addition there were at least two main lateral branches extending off the dorsal duct on each side: one, near the posterior end where the main duct bent mediad to unite with its opposite partner, and the other, part way up the body. The anterior lateral branch in turn subdivided, and tubules emanated from

the end of each subdivision. In one particularly clear specimen it appeared as though some of these tubules were directly continuous with the flame cells. There were as many as 230 flame cells in this plerocercoid, and apparently the tubules associated with the flame cells were concentrated mainly beneath the outer layer of calcareous corpuscles. The main dorsal duct had a rather constant diameter of about 8 μ, whereas the larger ventral duct varied from a maximum of 70 μ to a tubule which was completely constricted, the diameter depending on the activity of the plerocercoid. No current was observed in the dorsal duct, but several granules in the ventral duct moved back and forth following the contractions of the body.

The ventral ducts, showing no branches, bifurcations, or evidence of direct connection with the flame cells, extended further posterior than the dorsal ducts, and united in the vicinity of the excretory atrium. They did not unite directly, as described for *Paruterina*, rather forming a highly convoluted ring which surrounded the proximal end of the excretory atrium. In addition apparently two, and possibly as many as four, other small tubular connections, all lacking a pronounced sphincter, opened from the ventral excretory vessel into the excretory atrium. The rugae or papillae so prominent on the wall of the excretory atrium in *Paruterina* (6) were lacking in *C. globifera*. The six embryonic hooks were somewhat scattered and in the wall of the plerocercoid in the vicinity of the proximal end of the excretory atrium.

Natural infections with armed plerocercoids indistinguishable from those described above were found in Ontario in seven species of small mammals, namely: *Clethrionomys gapperi*, *Napaeozapus insignis*, *Peromyscus leucopus*, *P. maniculatus*, *Sorex cinereus*, *Tamias striatus*, and *Tamiasciurus hudsonicus;* all but *P. leucopus* were collected in Algonquin Park. These plerocercoids were slightly larger on the average than the specimens from experimental infections, the largest being 1.010 by 0.230 mm, although other specimens fell near the lower limit of the range recorded from experimental infections. The everted scoleces varied from 144 by 208 μ to 249 by 289 μ; the suckers from 69 by 69 μ to 104 by 90 μ; the diameter of the rostellar ring from 83 to 114 μ; the rostellar pad from 35 by 62 μ to 49 by 97 μ; and from 40 to 52 hooks, the large ones varying from 31 to 35 μ in length, and the small hooks from 21 to 27 μ in length.

The incidence of infection with plerocercoids of *C. globifera* is low since there were only 12 infected animals among the more than 2350 which were examined. There appears to be little host specificity, however, since 7 of the 18 species of potential hosts examined in Algonquin Park harbored this parasite. Likely more extensive surveys will reveal others as only 151 individuals of the remaining 11 species were examined in the present survey. The only infected host collected outside Algonquin Park (*Peromyscus leucopus* collected near Caledon East, Ontario) also came from a wooded area.

Armed plerocercoids which agreed completely with those described here as *C. globifera* were found twice in the liver of *Clethrionomys gapperi* collected

at the University of Minnesota Biological Station, Itasca Park, Minnesota, once by the author in 1948 and again in 1950 by the late Dr. Asa C. Chandler.

Metacestodes of species of *Cladotaenia* have been reported several times from the liver of various mammals in North America (1, 10, 13, 18, 24, 25, 27, 28, 31, 32), but some refer to *Taenia mustelae* Gmelin (5). There is a remarkable resemblance between the illustrations of proliferating cysticerci given by Penner (25) and *T. mustelae* identified by Freeman (5). Four whole mount preparations of metacestodes deposited at the University of Minnesota by Dr. Penner, and identified as *Cladotaenia* from a natural infection in the body cavity of *Microtus pinetorum*, proved to be typical uni- and multi-scolex cysticerci of *T. mustelae*.

Scott (31, 32) fed "single" and "multiple tapeworm cysts" from white-tailed prairie dogs to three, wild, nestling ferruginous rough-legged hawks. When killed later, two hawks had *C. globifera* and one had a mixed infection with this species and *C. circi*. It is evident, however, from Scott's feeding protocols provided by Mr. Honess (in litt.), that not all these worms could have resulted from the experimental feedings. In fact there is no way of knowing if any of the "multiple cysts" infected a hawk. This further supports the supposition that all records of proliferating metacestodes of *Cladotaenia* are misidentified cysticerci of *T. mustelae*.

Infecting the Definitive Host

Four separate hawks were fed several hundred plerocercoids of *C. globifera*. In all cases the results of the feeding were followed by examination of the feces for proglottides at weekly or more frequent intervals. For the first trial a juvenile goshawk received plerocercoids from natural infections in a *Napaeozapus insignis* and *Tamias striatus*, but the bird harbored no cestodes when killed and examined 46 days later. Later no helminths were recovered from a juvenile sharp-shinned hawk that died 6 months after it was fed plerocercoids from a wild *Clethrionomys gapperi*. Approximately 60 plerocercoids, many of which were alive, were recovered from feces passed by this hawk within 24 hours after the infectious meal. In the third feeding, plerocercoids which were 21 or 22 days old were fed to a second young sharp-shinned hawk. When killed 65 days later this bird harbored three small worms in the earliest stages of strobilation. In the final feeding a third young sharp-shinned hawk first was fed plerocercoids which were 27 days old, but no proglottides were passed in the feces during the next 10 months. At this time a second feeding of plerocercoids which were 343 days old was made, and again no proglottides were passed in the feces for the next 9 weeks at which time the experiment was terminated without killing the bird.

All birds which were fed plerocercoids were wild young hawks. The goshawk had a nest mate which died earlier and this bird harbored a natural infection of nearly mature *C. globifera*. The cestode eggs which produced the experimental plerocercoids which were fed to the sharp-shinned hawks came from another sharp-shinned hawk. Therefore experimental infections should have become established in both hosts. Similar difficulties have

been encountered in other attempts at establishing cestode infections in other carnivores, particularly the hypertensive forms. The fact that 60 plerocercoids were passed through one bird within the first day post-feeding indicates that intestinal emptying time may be important at least in some cases. The three small worms recovered in one sharp-shinned hawk 65 days post-feeding could have resulted from the experimental feeding. However, previous experience with other species of cestodes in carnivores (e.g., *T. mustelae* and *Mesocestoides* in mustelids) indicates that such non-strobilated cestodes may be "immature" morphologically, but as much as a year old chronologically; therefore this feeding is inconclusive.

The Life History of *Cladotaenia circi* Yamaguti

The development of the plerocercoid of *Cladotaenia circi* was followed in rodents that were fed eggs from ripe proglottides collected from a marsh hawk. Apparently many eggs were not infective, because only one of 10 *Mus musculus* and one of five *Peromyscus maniculatus* were infected, therefore, the egg shown in Fig. 12 may not be fully mature. Extensive lesions were present in the liver of the *M. musculus* when examined 14 days post-feeding. The lesions were diffuse and the parasites were difficult to locate. Possibly the plerocercoids were not within the lesions as occurred with infections of *C. globifera* of comparable age. The lesions in the *P. maniculatus* killed 20 days post-feeding were more circumscribed, although only three metacestodes were recovered.

These plerocercoids are similar in basic morphology to *C. globifera*. One living plerocercoid, which measured 1.45 mm in length and 0.40 mm in width at the scolex, had fully developed suckers, excretory system, and rostellar hooks after 14 days of development in *M. musculus* (Fig. 18), but one of the three plerocercoids in the *P. maniculatus* was not fully developed after 20 days development. The transparent cuticle on the 20-day-old plerocercoids was noticeably thicker than on the younger worms. All the plerocercoids quickly evaginated their scoleces when removed from the liver (Fig. 18), but the older ones reinvaginated before fixation. In the plerocercoid in Fig. 18 some bubbling of the cuticle and a partial prolapse of the excretory atrium occurred before the photomicrograph was taken.

The following measurements of fully developed plerocercoids are from five stained whole mounts. The length and width of two plerocercoids with evaginated scoleces were 0.980 by 0.300 mm and 1.010 by 0.245 mm, and the three with invaginated scoleces varied from 0.530 by 0.215 mm to 1.040 by 0.390 mm. The scoleces measured from 240 to 300 μ in width. The suckers varied from 69 by 56 μ to 104 by 83 μ, others were subspherical varying from 72 to 76 μ in diameter. The rostellar ring varied from 71 to 82 μ in diameter and the rostellar pad from 31 by 57 μ to 49 by 69 μ. There were from 54 to 60 hooks, the large ones from 23 to 24 μ in length and the smaller ones 20 to 21 μ in length (Fig. 19). A lacuna was lacking in the medullary parenchyma.

The excretory system in plerocercoids of *C. circi* was as described above for *C. globifera*, although not as much detail was seen. The dorsal duct was a simple tube lacking bifurcations and no auxiliary tubules branched from it. The ventral duct was a simple tube except that it formed a highly irregular but closed ring around the upper end of the excretory atrium. In a sectioned specimen at least one minute tubule, without a sphincter, united this ring to the atrium.

Apparently *C. circi* is rare or does not occur in Algonquin Park, since it was not encountered during the autopsy of more than 2350 small mammals and 7 hawks. Only a few rodents have been examined from elsewhere in the province, which may account for metacestodes of this species not being found in natural infections in Ontario. Mr. John C. Frandsen of the University of Utah has collected two species of armed plerocercoids in *Peromyscus maniculatus sonoriensis* in the mountains of Utah (in litt.). The morphology and measurements of a series of mounts of one species were indistinguishable from *C. circi* as described here, except that the maximum size of the large rostellar hooks was 25 μ rather than 24 μ.

Markowski (17) infected laboratory-reared *Mus musculus* on two occasions by feeding eggs of what he called *Cladotaenia cylindracea* (Bloch) from *Buteo buteo* collected in Europe. He also reported on metacestodes from a natural infection in *Mus agrarius* Pall. In a table he stated that rostellar hooks on the metacestodes from *M. agrarius* were 24 and 18 μ in length, whereas the hooks on the worms from *M. musculus* were 23.2 μ in length in one case and 29.0 μ in another, presumably all measurements being from sectioned material. Yet the hooks which he figured (presumably from *M. musculus*) measured 25 μ and 18 μ respectively. This indicates Markowski had more than one species, and one of them would appear to be *C. circi* as identified in the present study. It is of particular interest that Markowski's drawing of a free plerocercoid is similar to Fig. 20 of *C. globifera*, described above, which superficially resembles a cysticercus with an everted scolex. The limited number of plerocercoids of *C. circi* recovered during the present study precluded feeding them to hawks.

How to Differentiate Plerocercoids of *Cladotaenia* from *Paruterina*[8]

From this and a previous report (6) it is evident that the pleroceroids of *Cladotaenia* and *Paruterina* found in the liver and mesenteric lymph glands of various small mammals are very similar. Usually *Paruterina* is larger, but there is an overlap in the range of some measurements. Generally speaking the length of the guard on the rostellar hooks, measured from the top of the blade to the tip of the guard (*cd* in Fig. 1), is relatively longer in species of *Cladotaenia* than in *Paruterina*. Thus the ratio *ab*:*cd* (Fig. 1) appears to be a reliable index. In all of the writer's experience this ratio for

[8]In the collection of metacestodes from *Peromyscus maniculatus sonoriensis* from Utah submitted by Mr. Frandsen were some plerocercoids of *Paruterina candelabraria* (Goeze, 1782). This is the first record of a natural infection with the plerocercoid of this species.

Cladotaenia was less than 3.5:1 for the large hooks, and averaged less than 3.5:1 for the small hooks, whereas in *Paruterina* it was 3.5:1 or more, averaging near 4.0:1. Computation of these ratios from published records is hazardous, however, because the illustrations or measurements may not be from hooks which are in full side view. Even a slight tilting will drastically affect these proportions. The most reliable measurements are from unfixed specimens in wet mount, or permanent mounts of hooks which are placed into side view before fixation.

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SENSITIZATION TO MOSQUITO BITES[1]

J. A. McKiel[2]

Abstract

Laboratory animals can be sensitized to mosquito bites by injections of mosquito extract or by repeated exposures to mosquito bites. The immediate reaction occurs only in sensitized animals and therefore is an allergic manifestation. The delayed reaction also appears to have a similar cause. Mosquito extracts are not toxic to animals. The sensitizing material first appears in the life cycle of the mosquito late in the series of changes which take place within the pupa. This material is limited to the head–thorax region of the mosquito, presumably to the salivary glands, and probably is composed of more than one antigenic fraction.

Introduction

In spite of increased use of the newer insecticides, development of more effective repellents, and protective clothing, biting flies continue to be a serious problem in many parts of the world. Control is seldom completely successful; repellents are effective for relatively short periods of time; protective clothing has limited value particularly where such clothing may become torn or where normal activity requires exposure of parts of the body.

In recent years, areas of northern Canada have assumed increasing importance because of their natural resources and because these areas occupy a strategic position in continental defence. Here, biting flies, particularly mosquitoes and black flies, occur in dense hordes. Complete avoidance of bites is impossible and in sensitive individuals, reactions to bites may cause serious discomfort. Studies of reactions to mosquito bites have been part of a broad program of biting fly studies in Canada in recent years (18, 19).

This paper reports the results of studies in which use is made largely of laboratory animals to clarify the cause of reactions to mosquito bites, to investigate the occurrence in the mosquito of the substance or substances responsible for the reaction, and to study the degree of specificity exhibited by the reaction.

There is a similar pattern of reaction in humans to bites of all blood-sucking insects. Reactions to mosquito bites may be classed as immediate or delayed depending upon the time of onset of symptoms. An immediate reaction begins to appear within a few minutes after the bite. It consists of a soft, whitish wheal, usually surrounded by erythema, and accompanied by pruritus. Usually it disappears completely within an hour. A delayed reaction typically appears several hours after the bite and consists of a rather hard, raised lesion or papule. Frequently the papule is accompanied by erythema and an extremely intense pruritus. A delayed reaction may wax and wane many times for several days, often causing insomnia. Either or both of these reactions may develop, depending upon the individual's susceptibility.

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Little use has been made of laboratory animals in studies on reactions to insect bites. Prior to the present studies the writer found only one published report of animals having been sensitized experimentally to insect bites. According to that report, Dubin *et al.* (4) were able to sensitize rabbits to bites of *Anopheles quadrimaculatus* Say by means of three weekly subcutaneous injections of a suspension of triturated *A. quadrimaculatus*. Rabbits which had not been injected with mosquito antigen did not react when bitten by this mosquito. For studies on reactions to mosquito bites, laboratory-reared animals offer such advantages as absence of reaction to initial bites, ready availability, ease of handling, and a known history of exposure to mosquitoes. It is unfortunate that all of these conditions cannot be fulfilled when human beings are used as experimental subjects. A disadvantage of animals for this purpose is that they fail to develop delayed reactions to mosquito bites.

Although animals have been regarded generally as being difficult to sensitize to insect bites (7), no difficulty was encountered during the present study in sensitizing rabbits and guinea pigs to bites of mosquitoes. Sensitivity was produced by (*a*) exposures to large numbers of bites, and (*b*) by injections of mosquito extracts.

Methods

Sensitization by Repeated Exposure to Mosquito Bites[3]

The method of repeated exposures to bites was used successfully in sensitizing rabbits and guinea pigs. Several white rats were also sensitized by this method. Prior to exposure of an animal, the hair was clipped from an area of the hip and the short hair remaining was removed with a depilatory. Usually the hair did not grow back for several weeks. The exposures were begun by allowing two to four mosquitoes to engorge on the depilated area in order to ascertain that the animal was normally non-reactive. Following the preliminary test, the animal was placed inside a cage containing a large number of mosquitoes. Each exposure lasted 2 hours and was repeated each day for 5 days. It was not necessary to restrain the movements of rabbits because of their docile nature. Guinea pigs and rats were placed in individual wire-mesh cages, just large enough to accommodate the animals, and were exposed to mosquito bites continuously for 5 days. Food and water were made available to each animal.

Sensitization by Injection of Mosquito Extracts

This method of producing sensitization in animals was more readily carried out and usually resulted in the development of a higher level of sensitivity based on size of wheal.[4]

[3]Colonies of *Aedes aegypti* (L.), *A. atropalpus* (Coq.), and *Culex pipiens* (L.) were maintained at the Defence Research Kingston Laboratory. *A. vexans* (Meig.) and *C. restuans* Theo. were reared to adults from larvae collected in nature. Other mosquito species used in this study were collected by light-trapping and sweeping.

[4]In more recent work arising from the studies reported herein it has been found that sensitivity can be developed most consistently by the administration of two bites per day (11).

Rabbits were sensitized to *A. aegypti* bites by five daily injections of 1 ml of *A. aegypti* extract. They were tested for sensitivity 10 to 14 days after the final injection by allowing two or more mosquitoes to feed on the depilated area of the skin. Guinea pigs were sensitized by intraperitoneal injections.

Preparation of Mosquito Extracts

Mosquitoes were killed by ether or chloroform and weighed. Unless otherwise stated, males as well as females were used. They were homogenized in sufficient phosphate-buffered physiologic saline, pH 7, to give a 1:10 (w/v) suspension using a Waring blendor. Merthiolate was added to give a final concentration of 1:10,000. After storage for 48 hours at 6° C, the suspension was centrifuged at 2500 r.p.m. for 30 minutes. The supernatant was removed and sterilized by Seitz filtration.

Reactions of Animals Sensitized to Mosquito Bites

The reactions to mosquito bites in animals, previously sensitized either by repeated exposures to bites or by injections of extract, differ in certain respects from that of sensitive human subjects. The erythema, frequently so striking in the sensitive person, is nearly absent in the animal. Also, the wheal is slower in making its appearance and likewise slower in disappearing. Although subject to variation, the wheal in the rabbit, guinea pig, or rat begins to appear in about 15 minutes and reaches a maximum in about 1 hour, then begins to flatten, and the border becomes less distinct. For this reason, the diameters of wheals in animals were measured and recorded 1 hour after the bites were received.[5] As the wheal begins to flatten, its color changes from white to reddish. The wheal may disappear entirely within 8 hours or assume a papular appearance and persist for as long as 2 or 3 days. Delayed reactions, distinctly separated from the immediate reaction, have not been observed in animals in response to mosquito bites.

In sensitive animals (as well as in sensitive persons) a shallow probe of the proboscis is usually sufficient to cause the formation of a wheal. Size of the wheal is usually greatest when the mosquito has had difficulty in locating a blood source and has had to make several partial withdrawals and penetrations before obtaining blood. This is to be expected since, presumably, a secretion is injected during the whole probing process. To minimize variations originating from this source, mosquitoes were allowed to engorge fully as a standard procedure in testing reactions to mosquito bites.

The Cause of Mosquito Bite Reactions

Toxicity as a Cause of Bite Reactions

Several references in the early literature suggest that reactions to insect bites are due to injected toxic material. Hirschfelder and Moore (10) and McKinley (13) regarded the secretion injected by mosquitoes as a poison which

[5]This procedure followed the practice adopted by Last and Loew (12), who found that the 60-minute observation was the most satisfactory method for recording intradermal reactions of rabbits to a variety of agents.

may be irritating to human skin and to the skin of animals. Extracts of salivary glands of *A. aegypti* produced no effect, however, when injected intracutaneously into guinea pigs. Gordon and Crewe (7) were unable to find any evidence by gross examination that any reaction occurred in animals following first exposure to mosquito bites. In contrast, Rockwell and Johnson (16) reported that immediate and delayed 2- to 4-mm papules developed in two rabbits with no previous exposure to mosquitoes, and that mosquito extracts were sufficiently toxic to cause death when injected into guinea pigs.

During the present study more than 200 rabbits and 50 guinea pigs with no previous exposure to mosquitoes were tested for reactions to *A. aegypti* bites and injected with 1:10 (w/v) mosquito extracts. No reactions were detected in any of these animals as a result of the bites, nor were there ever any indications of toxic symptoms attributable to the extracts. However, these extracts were prepared from freshly killed mosquitoes, whereas Rockwell and Johnson prepared their extracts from dried mosquitoes. The possibility that dry mosquitoes may contain toxic substances was tested in the following manner.

Since it was not known how long and to what degree the mosquitoes used by Rockwell and Johnson had been dried, one extract was prepared from mosquitoes which had been dried to constant weight in a desiccator jar under vacuum. A second extract was prepared from mosquitoes which had been permitted to dry at room temperature in an open jar for 6 months. Rockwell and Johnson's method of preparing the extract was followed, using Coca's solution as the extractant.

Each extract was tested for toxicity by injecting 0.2 ml intracutaneously into five 250-g guinea pigs which had never been exposed to mosquitoes. None of the 10 animals showed any toxic effects during a 2-hour period after the injection and all were healthy 2 weeks later.

These results do not confirm Rockwell and Johnson's conclusion that mosquito extracts are toxic to animals.

Histamine in the Mosquito Saliva as the Cause of Immediate Bite Reactions

It is well known that histamine, if injected into a person's skin, will cause a reaction similar in appearance to an immediate reaction resulting from a mosquito bite. Eckert *et al.* (5, 6) used *C. pipiens* to compare the histamine content of unfed mosquitoes with that of mosquitoes which had been allowed to probe human skin without obtaining blood. The weight loss of histamine during probing was computed to be about 0.05 μg per probe per mosquito. The authors concluded that the immediate reaction to mosquito bites is caused by histamine which the mosquito injects while feeding and that variations in the reaction depend upon the person's susceptibility to histamine.

The fact that the skin of normal laboratory rabbits does not react to mosquito bites but can be made reactive by injections of mosquito extract would imply, if Eckert *et al.* are correct, that the bite-reactive rabbits had become sensitized to histamine. To test this possibility, 10 *aegypti*-sensitive rabbits (5- to 7-mm wheals elicited by *A. aegypti* bites) and 10 normal rabbits (no

reaction to *A. aegypti* bites) were injected intracutaneously with 0.05 and 5.0 μg of histamine diphosphate. Neither group of animals developed true wheals at any of the injected sites.[6] Further evidence against histamine as the direct cause of mosquito bite reactions in sensitive rabbits was provided by the following experiment.

Thirty milliliters of a 1:10 *A. aegypti* extract were placed in a cellophane tube and dialyzed at 6° C against 20 l. of flowing distilled water for 48 hours. The extract was then passed through a Seitz filter and injected subcutaneously into four rabbits and intraperitoneally into three guinea pigs. All of these animals developed wheals when later exposed to *A. aegypti* bites, demonstrating that the sensitizing material is non-dialyzable. Since histamine dialyzes freely through cellophane, it could not have played any part in the sensitization process.

A study to be reported in a subsequent paper repeatedly demonstrated that human subjects sensitive to *A. aegypti* bites will react when skin-tested with the non-dialyzable portion of the mosquito extract. For these reasons it is concluded that the immediate reaction to mosquito bites cannot be attributed to histamine as a component of the secretion injected by the mosquito.

Allergy as the Cause of Bite Reactions

Although Rockwell and Johnson (16) regarded toxicity as a contributory cause of reactions to mosquito bites, these authors believed that allergy plays a more important role. Indeed, allergy has received rather wide acceptance in recent years as the explanation of the cause of the immediate reaction to insect bites.

The following observations on reactions of a child to mosquito bites supports this theory.

The child was first bitten by *A. aegypti* when he was three years old. Previous exposures to mosquitoes was known to have been light. On first exposure to *A. aegypti* the child received five bites on the left forearm. This arm was observed closely for 2 hours and no reaction developed. The right forearm was then exposed and it received 48 bites. No reaction developed during the ensuing 7 hours. However, 21 hours after the first bites were received, 1–2-mm papules were present on both arms and were accompanied by considerable erythema. On the fifth day the erythema disappeared. On the eighth day the lesions lost their papular appearance but persisted several days longer as brownish spots. Pruritus was not present at any time during the course of the reaction. Nineteen months later (during which the child had had two seasons' exposures to indigenous mosquitoes), he was exposed again, this time to three *A. aegypti* bites. For the first time, a distinct immediate reaction developed consisting of wheals 2.6, 5.5, and 4.7 mm in diameter without erythema and pruritus. Twelve hours later, a delayed reaction characterized by inflamed papules 9–15 mm in diameter was present. Pruritus was very intense and lasted for 8 hours. Non-itching papules persisted for 2 days.

[6]The skin of rodents and rabbits is refractory to histamine according to Darsie *et al.* (2) and Last and Loew (12).

This case history exemplifies the development of an allergy of the immediate type which clearly was acquired as a result of exposures to mosquito bites.

These observations also illustrate the increase in delayed symptoms commonly noted in young children after successive exposures to indigenous mosquitoes. Although the delayed reaction has been attributed to toxic properties of the mosquito's salivary secretion by a number of writers including Gordon and Crewe (7), it is difficult to understand how the toxicity theory can account for the increased severity of the reaction as described above. Rather, one would expect that, during a series of exposures, the reactions to a toxin either would remain approximately constant or would decrease in size as a result of an acquired immunity to the toxin. This trend towards an increasingly severe delayed reaction is explained more logically on the basis of a developing allergy, as was suggested by Theodor (17) and Heilesen (9).

The Sensitizing Material of the Mosquito

First Appearance of Sensitizing Material in the Stages of the Mosquito's Life Cycle

The adult mosquito already has been shown to possess antigenic material which is capable of sensitizing animals to bites of that mosquito. The following experiment was made to determine whether the sensitizing substance is peculiar to the adult mosquito. For this purpose, extracts were prepared of *A. aegypti* eggs, fourth instar larvae, 24-hour-old pupae, and fully mature pupae. Each of the four extracts was tested in three guinea pigs. None of the guinea pigs injected with extracts of eggs, larvae, or 24-hour pupae became sensitized to *A. aegypti* bites. However, one of three animals injected with the extract of mature pupae developed sensitivity to mosquito bites. The results show that the allergenic materials is not present in eggs, in larvae, or in young pupae. Its presence in the mature pupal extract shows that the sensitizing substance develops during the late stages in the transformation of the mosquito from larva to adult.

Presence of the Sensitizing Material in Male Mosquitoes

It was recognized early in this study that large amounts of mosquito extract would be required and that full use should be made of the mosquitoes reared. The fact that male mosquitoes do not take blood meals suggested the possibility that they may not contain the bite-sensitizing material present in females. To resolve this question, a 1:10 extract was made of male *A. aegypti* mosquitoes and was injected into four normal rabbits. When later tested for reactions to *A. aegypti* bites, all four rabbits developed wheals at the bitten sites, demonstrating that the sensitizing substance is common to both sexes of the species.

Origin of the Sensitizing Substance in the Adult Mosquito Body

Several workers have produced skin reactions in sensitive persons by means of extracts not only of the salivary glands but also of various other parts of the mosquito. Rockwell and Johnson (16) found that skin reactions were elicited by higher dilutions of *A. aegypti* abdomen extract than by head–

thorax extract. These results suggested that the sensitizing substance is present throughout the mosquito body with the highest concentrations being in the abdomen. However, it has been pointed out by Dawson (3) that compounds which are active in eliciting skin reactions in sensitive persons are not necessarily active in inducing sensitization.

In order to determine whether the sensitizing substance is present throughout the mosquito body, approximately 7500 female *A. aegypti* were cut with a razor blade so as to separate the head–thorax region from the abdominal region of each mosquito. Standard 1:10 saline extracts were prepared of the two portions. Each extract was tested first to determine whether it was capable of eliciting skin reactions. To this end, two guinea pigs and one rabbit, previously sensitized by exposures to large numbers of *A. aegypti* bites, were injected intracutaneously with 0.01 ml of each extract. Saline controls were included in the test. Positive reactions developed at the sites injected with the extracts. The head–thorax extract was then injected subcutaneously into four normal rabbits and intraperitoneally into four normal guinea pigs. An equal number of animals was injected similarly with abdomen extract. When these animals were exposed 13 days later to *A. aegypti* bites, the four guinea pigs and three of the four rabbits previously injected with head–thorax extract developed 5- to 7-mm wheals. None of the animals injected with abdomen extract developed any reaction to the bites.

The four rabbits which had received the subcutaneous injections of the abdomen extract and the one non-sensitive rabbit of the group injected subcutaneously with head–thorax extract were given the standard course of injections of *A. aegypti* whole-mosquito extract. When again exposed to *A. aegypti* bites, all rabbits which originally had failed to become sensitized by means of abdomen extract now developed 6- to 7-mm wheals. However, the formerly non-sensitive rabbit of the group injected with head–thorax extract showed only a low level of reactivity to *A. aegypti* bites (5-mm wheals). It is concluded that this rabbit, unlike the four rabbits originally injected with abdomen extract, possessed a native resistance to mosquito bite sensitization.

The failure of rabbits and guinea pigs to react to mosquito bites after injections of abdomen extract indicates that the sensitizing antigen either is not present in the abdomen or is present in very small amounts. In view of Rockwell and Johnson's findings that the skin-reacting substance is present in greater concentration in the abdomen, it is concluded that the reacting substance of the abdomen will combine with preformed antibodies but is not capable of stimulating their production in the body of the host. In other words, the skin-reacting substance of the mosquito abdomen appears to be a hapten, while the sensitizing substance of the head–thorax region is probably a complete antigen. Since animals can be sensitized by mosquito bites and since the sensitizing substance is limited to the head–thorax region, the sensitizing substance must be associated with the part of the digestive system lying in the head–thorax region. Therefore, it may be deduced that the sensitizing substance forms part of the secretion of the salivary glands.

Specificity of Sensitization to Mosquito Bites

Heilesen (9) found that a large proportion of his subjects in Sweden reacted to *A. aegypti* bites even though these subjects had not been outside that country and therefore could not have been exposed previously to this mosquito. A similar observation was made by O'Rourke and Murnaghan (15) in Canada. Several workers have noted differences in reactions to bites of different species of mosquitoes. A subject reported by Brown *et al.* (1) developed less reaction to *Culex quinquefasciatus* Say and *Aedes taeniorhynchus* (Wied.) than to *A. aegypti*. Differences in reactions to bites of *Anopheles* and *Aedes* have been reported by Hecht (8), Mellanby (14), Heilesen (9), and Rockwell and Johnson (16). In addition Heilesen stated that one subject developed an immediate reaction to *A. aegypti* bites but no reaction when bitten by *Aedes albopictus* (Skuse).

Specificity Tests Made on Human Volunteers

In order to obtain additional information concerning the specificity of bite reactions, 10 subjects were exposed to bites of *A. aegypti* and to one or more of the following three species: *A. atropalpus*, *A. vexans*, and *C. pipiens*. Each subject received at least eight bites by each of the species tested. Immediate wheals and erythema were measured 30 minutes after the subject was bitten. Delayed reactions if present were recorded daily as +, ++, or +++. The results given in Table I show that 7 of the 10 subjects reacted differently when bitten by different species of mosquitoes and therefore exhibit some degree of specificity (exceptions being BD, BJ, and DA).

TABLE I

Comparison of reactions to bites of various species of mosquitoes

		Immediate reaction						Delayed reaction	
		Diameter of wheal (mm)			Diameter of erythema (mm)				
Subject	Mosquito	Mean	Min.	Max.	Mean	Min.	Max.	Degree at maximum	Duration of reaction
LA	*A. aegypti*	14.7	10.8	17.1	57	46	65	—	—
	A. atropalpus	9.9	7.8	11.4	43	35	48	—	—
TW	*A. aegypti*	10.6	9.9	11.3	44	38	50	—	—
	A. atropalpus	5.9	5.5	6.2	27	22	30	—	—
MA	*A. aegypti*	9.3	6.5	12.8	45	24	57	—	—
	A. atropalpus	3.8	3.6	4.4	27	21	36	—	—
	A. vexans	6.2	4.5	7.1	40	23	51	—	—
	C. pipiens	2.7	2.5	3.5	6	5	8	+	1 day
MP	*A. aegypti*	16.2	13.4	17.6	45	26	60	—	—
	A. atropalpus	10.2	6.8	12.8	34	27	41	—	—
BD	*A. aegypti*	6.9	4.9	7.7	0	0	0	—	—
	A. atropalpus	7.1	6.2	7.5	0	0	0	—	—
BJ	*A. aegypti*	4.1	3.0	4.8	0	0	0	—	—
	A. atropalpus	4.0	3.0	4.5	0	0	0	—	—
DA	*A. aegypti*	2.9	2.5	3.5	0	0	0	+	1 day
	A. atropalpus	3.1	2.5	3.4	0	0	0	+	1 day
HM	*A. aegypti*	3.6	3.0	4.1	0	0	0	+	1 day
	A. atropalpus	0	0	0	0	0	0	+	1 day
RD	*A. aegypti*	2.5	2.0	3.2	5	3	7	++	5 days
	C. pipiens	3.0	2.5	3.6	6	3	9	—	—
RP	*A. aegypti*	2.5	2.0	3.1	0	0	0	+++	10 days
	C. pipiens	2.6	2.0	3.4	5	3	8	—	—

Specificity Tests Made on Laboratory Rabbits

Studies on the specificity of bite reactions when carried out on human subjects are almost always handicapped by the subjects' varied and unknown histories of exposures to indigenous species of mosquitoes. The use of laboratory animals whose previous exposures to mosquitoes are known makes it possible to overcome this difficulty.

The rabbits used in this test were sensitized to the bites of one species by injections of an extract of that species (either *A. aegypti* or *A. atropalpus*) and were then allowed to be bitten by the test species. Each rabbit received from 3 to 20 bites by the species being tested. Of the results given in Table II, tests numbered 3, 6, 8, 9, 10, 11 were carried out at Churchill, Manitoba. The mosquitoes used in the remaining tests were either collected or reared at Kingston, Ontario.

Of the 14 tests shown in Table II, cross-reactions were observed in only five of them indicating, as already noted in tests on human subjects, that mosquito bite reactions generally exhibit some degree of specificity. It is apparent from Table II that the occurrence of cross-reactions cannot be accounted for on the basis of the subgeneric relationship of the mosquitoes involved. The observation that the salivary fluids injected by at least some species of mosquitoes cross-react suggests that these fluids may contain more than one fraction capable of eliciting reactions in sensitive skin.

TABLE II

Reactions to bites of several species of mosquitoes in rabbits sensitized with an extract of either *A. aegypti* or *A. atropalpus*

Test No.	Species used to sensitize rabbits	Species used to test for cross-reaction	No. rabbits tested	No. rabbits developing cross-reaction
1	*Aedes* (*Finlaya*) *atropalpus* (Coq.)	*A.* (*Stegomyia*) *aegypti* (L.)	4	2
2	*A.* (*S.*) *aegypti*	*A.* (*F.*) *atropalpus*	25	3
3	"	*A.* (*Ochlerotatus*) *campestris* D. and K.	5	0
4	"	*A.* (*O.*) *canadensis* (Theo.)	1	0
5	"	*A.* (*O.*) *excrucians* (Wlk.)	2	1
6	"	*A.* (*O.*) *excrucians*	4	2
7	"	*A.* (*O.*) *fitchii* (F. and Y.)	2	0
8	"	*A.* (*O.*) *flavescens* (Muller)	2	2
9	"	*A.* (*O.*) *hexodontus* Dyar	5	0
10	"	*A.* (*O.*) *nearcticus* Dyar	3	0
11	"	*A.* (*O.*) *nigripes* Zett.	2	0
12	"	*A.* (*Aedes*) *vexans* (Mg.)	5	0
13	"	*Mansonia perturbans* (Wlk.)	8	0
14	"	*Culex pipiens* (L.)	7	0

Summary

In the early literature, reactions to mosquito bites were interpreted as being caused by a toxic substance which the mosquito injected while taking a blood-meal. This explanation still finds its suppporters even in relatively recent

writing. Data obtained in this study, however, do not support the proposition that mosquitoes contain substances which are toxic even when an extract of whole mosquitoes is injected into laboratory animals. Furthermore, rabbits and guinea pigs bitten for the first time by mosquitoes were never observed to develop any skin reaction other than an occasional hemorrhage caused by mechanical injury. The suggestion that mosquitoes inject histamine into the wound and that this compound is responsible for the immediate bite reaction is untenable. Histamine is freely dialyzable yet dialyzed mosquito extract will cause skin reactions in human volunteers sensitive to mosquito bites. Also, dialyzed mosquito extract will sensitize laboratory animals to mosquito bites. The degree of specificity observed both in human volunteers and in animals argues against the possibility that bite reactions are caused by a single, simple chemical compound.

The fact that laboratory animals which normally do not react to mosquito bites can acquire a sensitivity to the bites by being bitten repeatedly or by being injected with a mosquito extract provides additional evidence that the immediate reaction is caused by an allergy to, rather than toxicity of, the fluid injected by the feeding mosquito. The sequential stages in the acquisition of mosquito bite sensitivity in children lend weight to this theory and also strongly suggest that the delayed reaction has a similar cause.

Extracts of the head–thorax and of the abdominal regions of the mosquito were shown to be capable of producing reactions in animals sensitized by exposures to mosquito bites. However, the abdomen, unlike that of the head–thorax region, failed to sensitize normal animals to mosquito bites. These observations are interpreted as indicating that the material in the abdomen which elicits reactions when injected intracutaneously is haptenic in nature whereas the active material of the head–thorax region is fully antigenic. Specificity studies suggest that more than one sensitizing antigen is present in the salivary secretion of the mosquito.

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THE PARASITISM OF BLACK FLIES (DIPTERA, SIMULIIDAE) BY LARVAL WATER MITES MAINLY OF THE GENUS SPERCHON[1]

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Abstract

Larval sperchonid hydracarina, possibly *Sperchon jasperensis* Marshall, parasitized adult simuliids in Canada. All stages of the water mites were found during the summer. Larval mites gained access to adult black flies as they emerged. Parasitism occurred from spring until fall, reaching a peak in July in Algonquin Park, Ontario, and in August at Baker Lake, N.W.T. Diurnal changes in parasitism followed the pattern of emergence of the infested simuliid species. Larval mites that had doubled in size as parasites regained the water as the female flies were ovipositing. Some simuliid species were more attacked than others, and females more than males, observations which appear related to the amount of nutrient stored in the fly. This nutrient may benefit the parasites directly, or benefit them indirectly because flies with more nutrient more quickly complete the adult phase of their cycle. The most important bloodsucking species of black flies were the least infested by sperchonid mites.

Introduction

Most water mites (Hydracarina) are parasitic as larvae and predaceous as adults. Those of the family Sperchonidae have been infrequently discussed in the literature. Lundblad (14, p. 372) stated that sperchonid larvae were first described and figured by Protz (20, pp. 24–25). Koenike (11, p. 127) recognized that the larval mites collected from *Orthocladius* chironomid larvae and pupae by Dr. Thienemann in 1904 (23, p. 301) and by Taylor (22) were in the genus *Sperchon* (14, 23). Münchberg (18) mentioned a few other papers on *Sperchon* including those of Potthast (19, p. 262), Walter (27), and Viets (26).

Several authors have referred briefly to the parasitism of simuliids by unidentified water mites (2, 3, 7, 8, 13, 24, 25). Webster (28) spoke of larval mites, *Limnesia* sp., from a female *Simulium* which was attacking a horse in Texas.

The study of the population of adult black flies emerging throughout the 1946–1947 seasons from a square yard of Costello Creek, Algonquin Park, Ontario (4), afforded a unique opportunity of determining, as well, the seasonal parasitism of different simuliid species by water mites. In addition the presence of larval water mites on aquatic pupae, on adults netted away from the stream, on ovipositing females, and on newly laid egg masses was studied. Counts were made also of the number of water mites infesting emergent black flies from Whitehorse, Yukon Territory; Baker Lake, North-

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west Territories; Churchill, Manitoba; and Goose Bay, Labrador—flies that I was identifying from the Defence Research Board of Canada and the Canada Department of Agriculture. These black flies had been collected periodically throughout the season by workers on a grant to Dr. F. P. Ide from the Board and by members of the staff of the Entomology Division of the Department under the direction of Dr. C. R. Twinn.

Identity of the Mites Attacking Black Flies

The larval water mites parasitizing black flies are difficult to identify to species. An adult mite from the same habitat as the parasitic larval mites (Costello Creek, August 9, 1947) was identified tentatively in 1950 by myself as being in the genus *Sperchon* Kramer. Since that time, Dr. L. J. Stannard (Illinois Natural History Survey) and Dr. R. D. Mitchell (University of Florida) have identified, as *Sperchon*, other adults that I collected in Algonquin Park at sites where black flies were often heavily attacked by larval mites. Dr. Paul Münchberg (18) has identified, as *Sperchon*, nymphs reared by him from larvae that I had collected from females of *Simulium decorum* Walker on June 10, 1955, just below Lake Sasajewun dam, Algonquin Park. He made drawings of both the larva and nymph (18, p. 366) and indicated a striking similarity between the epimeral plate of the latter and that of *Sperchon jasperensis* Marshall (16). Mitchell (1957, personal communication) considered that all the *Sperchon* adults collected from Costello Creek and North Madawaska River just below Lake Sasajewun dam might be *S. jasperensis* or a species close to it. Larval mites on simuliids from Churchill were identified as *Sperchon* and the similarity of the larval water mites taken at Whitehorse and Baker Lake to those from Algonquin Park suggests that these too are in the genus *Sperchon*.

Dr. Mitchell identified single adult mites of two other genera that I collected in Algonquin Park, *Atractides* sp. (Atractideidae), from a grassy riffle below Lake Sasajewun dam and just above a pond-like widening of North Madawaska River, and *Tyrrellia ovalis* Koenike (Limnesiidae), from a small outlet in a marshy pond, 1 mile from Lake Sasajewun. There is no evidence, however, that these attack black flies.

Another larval mite larger, hairier, and rosier-orange than *Sperchon*, with a more elongate cephalic region, occurred occasionally on adult black flies in Algonquin Park and at Goose Bay, Labrador. It bore two red dots on the anterior sides of the soft pleura, above and anterior to the second legs. It is in the family Trombidiidae (Mitchell, 1957 (personal communication)). Thus this mite probably gained access to the flies when they settled near the ground and not as they emerged.

Species of Black Flies Attacked

Algonquin Park

Seven species of black flies were parasitized by larval water mites of the genus *Sperchon* during the summer of 1947 in Algonquin Park (Table I).

TABLE I

The infestation of different species of black flies emerging from Costello Creek. Included below are only those flies emerging during the infestation period, from June 27 to August 22, 1947, although emergence continued from May 28 to August 27

Species	Males			Females		
	Total no. mites	Total* no. flies	Average† mites per 100 flies	Total no. mites	Total* no. flies	Average† mites per 100 flies
C. dacotensis (D. & S.)	7	19 (11)	37 (5)	29	7 (86)	410 (10)
C. mutata (Malloch)	0	0 (0)	0 (0)	0	5 (0)	0 (0)
S. aureum Fries	272	105 (56)	260 (16)	1,193	175 (86)	680 (20)
S. decorum Wlk.	927	3,585 (18)	26 (12)	14,334	3,088 (76)	460 (32)
S. latipes Meigen	120	41 (56)	290 (14)	434	81 (78)	540 (20)
S. tuberosum Lund.	48	207 (9)	23 (3)	32	149 (10)	21 (4)
S. venustum Say	323	7,649 (3)	4 (5)	4,248	15,779 (15)	27 (18)
S. vittatum Zett.	260	1,751 (9)	15 (9)	1,195	1,694 (34)	70 (15)
Total	1,968	13,499 (8)	15 (16)	21,465	20,973 (26)	100 (32)
Average for both sexes (mites per 100 flies) = 68						

*Percentage of flies infested, in parentheses.
†Maximum number per fly, in parentheses.

In four species over 75% of the females were attacked whereas only in two species were more than 50% of the males attacked. In the heavily parasitized species there were from 400 to 700 mites per 100 females whereas for all species and sexes the average was only 68 per 100 flies. Both sexes of *Simulium aureum* Fries and *S. latipes* Meigen[3] were attacked by the largest number of mites, the females having two to three times the number per fly as the males. *Cnephia dacotensis* (Dyar and Shannon) and *Simulium decorum* were the next most heavily infested, especially the females. *Simulium venustum* Say, the commonest blood-sucking simuliid over much of Ontario, was among the least attacked (15% of the females), there being only 27 mites for every 100 females on the average. No mites were seen on *Cnephia mutata* (Malloch) nor on *Prosimulium hirtipes* (Fries)[4] but the emergence of the latter had ended before the attack of *Sperchon* began. In 1946 the same species, except for *C. dacotensis*, which was not present, were the most heavily infested and *S. venustum* the least. Chironomids were also found to be infested with *Sperchon* and possibly hygrobatid larvae (determined by Mitchell, 1957) but the number of mites per 100 midges was only 25 in 1947. Other aquatic insects such as Plecoptera, Ephemerida, and Trichoptera were not parasitized in 1946 and 1947, although they emerged in large numbers.

A larva of the larger trombidiid mite was seen on the hind tibia of a female of *P. hirtipes* (s.l.) collected on June 5, 1939, from Costello Creek, and one on the dorsum of the hind abdomen of a female *S. venustum* on May 27, 1944. One was found on the mesopleuron of a female *S. venustum* on June 11, 1955, one on the dorsum of the seventh abdominal segment of a female of *S. decorum* on June 14, 1955, and one on a female *S. decorum* on July 7, 1955, all netted

[3]The identity of adults of this species without their exuviae and cocoons is only tentative.
[4]This is either one or both of *P. fuscum* Syme and Davies and *P. mixtum* S. and D., new species recently described (22).

TABLE II

The period of water mite attack on black flies (species indicated) during the summer of 1948 at different stations where emergence collections were made at Churchill, Manitoba

Station	Collection period	Period checked for mites	Period of mite infestation	Species attacked
No. 1. Radio Tower stream	June 26 to August 20	June 27 to July 27	July 16 (1 mite)	*S. aureum* female
No. 2. Water Tower stream, from Lake Isabelle to Churchill River	June 27 to August 20	June 27 to August 20	July 10 to August 11	*C. dacotensis* *S. furculatum* *S. decorum* *S. venustum*
No. 3. Hospital stream	August 13 to 20	August 13 to 20	None	None
No. 4. Hospital stream	June 27 to August 4	June 27 to July 28	July 20 to 28	*S. aureum* *S. decorum* *S. venustum*
No. 6. Goose River	June 28 to July 22	June 28 to July 22*	None	None
No. 7. Goose River	June 26 to August 20	June 29 to August 20	None	None

NOTE: *Not checked from June 30 to July 17.

over the North Madawaska River. Single specimens of the same type appeared in 1955 on a female of *Tabanus nivosus* O.S. and on one of *T. trepidus* McDunnough.

Goose Bay, 1949

No mites were observed on black flies that emerged from the "American Pump House" stream, but no *S. aureum* and *S. decorum* adults occurred in emergence collections (10). None were seen on specimens reared from the Insectary, Mid Terrington and South Terrington streams. The only two larval mites, the larger trombidiids seen in Algonquin Park, occurred in a netting collection on August 13, near the Insectary stream. One was attached to a female of *S. latipes* and the other was loose in the collecting bottle. In 1950 in netting collections of mainly *P. hirtipes* (s.l.) and *S. venustum*, six to eight mites were found on a total of 7000 flies (Hocking, 1951 (personal communication)).

Churchill, 1948

Black flies collected during the summer from six emergence cages were examined for parasitism by sperchonid larvae (Table II). Mites were found on flies from three of the cages. In the Hospital stream draining into the east side of Churchill River, 5 miles from its mouth, *S. aureum* and *S. decorum* were again the most heavily infested (Table III). The average number of mites per adult of *S. decorum* and *S. venustum* was much greater than that found in Algonquin Park in 1947, but the males of these two species were more heavily infested than the females. In the Water Tower stream, draining into the east side of the River, 1 mile north of Hospital stream, the infestation on *S. decorum* was lower than that on *S. venustum* (Table IV). This is explained by the greatest emergence of *S. decorum* occurring before and after the peak of the mite population. At this station *S. furculatum* Shewell was also infested.

TABLE III

The infestation of different species of black flies emerging from Hospital stream (station No. 4), Churchill, Manitoba, from July 20 to 28, 1948. Some collections made during the period of infestation (i.e. July 29–August 4) were not checked for mites and these are not included in the figures; thus the infestation may have continued into August as it did at station No. 2

	Males			Females		
Species	Total no. mites	Total no. flies	Average* mites per 100 flies	Total no. mites	Total no. flies	Average mites per 100 flies
S. aureum Fries	76	20	380	51	8	640
S. decorum Wlk.	27	2	1,350 (17)	15	2	750
S. venustum Say	80	34	240	81	91	89
Total	183	56	330	147	101	150
Average for both sexes (mites per 100 flies) = 210						

*Maximum number of mites per fly, in parentheses.

TABLE IV

The infestation of different species of black flies emerging from Water Tower stream (station No. 2), Churchill, Manitoba, from July 10 to August 11, 1948. A number of collections made during this period (July 9, 11–16, 18–26) were not checked for mites and they are not included in the figures

Species	Males			Females		
	Total no. mites	Total no. flies	Average mites per 100 flies	Total no. mites	Total no. flies	Average mites per 100 flies
C. dacotensis (D. & S.)	0	2	0	1	3	33
S. decorum Walker	20	146	14	12	182	7
S. furculatum Shew.	3	1	300	0	0	–
S. venustum Say	15	22	68	31	93	33
Total	38	171	22	44	278	16

Average for both sexes (mites per 100 flies) = 18

TABLE V

The infestation of different species of black flies emerging from stream at station No. 1, Baker Lake, Northwest Territories, from August 11 to 17, 1949. Collections of simuliids were made from July 24 to August 19

Species	Males			Females		
	Total no. mites	Total* no. flies	Average† mites per 100 flies	Total no. mites	Total* no. flies	Average† mites per 100 flies
Cnephia sp. X	309	1,237 (14)	25 (12)	864	709 (46)	120 (11)
C. emergens Stone	0	35 (0)	0	0	18 (0)	0
S. furculatum Shew.	3	2 (50)	150 (3)	2	11 (9)	18 (2)
S. tuberosum Lund.	12	5 (20)	240 (12)	0	1 (0)	0
Total	324	1,279 (14)	25 (12)	866	739 (46)	120 (11)

Average for both sexes (mites per 100 flies) = 61

*Percentage of flies infested, in parentheses.
†Maximum number of mites per fly, in parentheses.

Baker Lake, 1949

At station No. 1, on a small stream flowing into the northwest corner of Baker Lake, a species of *Cnephia*[5] (possibly the *borealis* referred to by Malloch (15)) was the most abundant species (Table V). The females were five times as heavily infested with mites of the *Sperchon* type as the males. The males of *S. furculatum* and *S. tuberosum*, though in low numbers, were more heavily attacked than the females. *C. emergens* was not infested. At station No. 2, on a small stream flowing into Baker Lake, 4 miles west of station No. 1, no mites were seen from August 1 to 17 on the emergent adults which were mainly *C. emergens* and a few *S. tuberosum*.

[5]Referred to as *Cnephia* sp. 'X' in an unpublished report to the Division of Entomology at Ottawa by D. M. Davies in 1950.

Whitehorse, 1949

Emergence collections from the Lewes River, Large Macrae, Little Macrae and Porter creeks, and a stream at mile 906 of the Alaska Highway were examined and no mites were seen on newly emerged black flies. Two mites of the *Sperchon* type were seen on the lower side of the thorax of a chironomid that emerged from Little Macrae creek in the first week of September.

Simuliid Species Attacked and the Amount of Stored Nutrient

Why were some simuliid species more heavily parasitized by water mites than others? That a chemical stimulus attracts the larval mites to the host has yet to be established. Whatever stimuli draw the larval mites to the host, these stimuli result in the greatest parasitism occurring on the females of those simuliid species with moderate to large fat bodies or almost mature eggs (Table VI). Females of *S. venustum* and *S. tuberosum* with small fat bodies on emergence from the pupa are much less attacked than females of *S. aureum* and *S. decorum*. However, females of *S. vittatum*, although having about as much stored nutrient as *S. aureum*, were only about 1/10 as infested with water mites (Table I), for which no explanation was found. That the females usually have more stored nutrient than males may also account for the former being more heavily parasitized.

TABLE VI

The average infestation of larval water mites on newly emerged female black flies, and the size of the fat body, based on collections from Costello Creek, 1947

Species	Number of flies	Average no. mites per 100 flies	Proportion of abdominal space filled with fat body
S. aureum	175	680	6/8–7/8
S. latipes	81	540	1/2 or less
S. decorum	3,088	460	All but for the small ovary
C. dacotensis	7	410	Little fat body, but almost mature eggs fill abdomen
S. vittatum	1,694	70	6/8–7/8
S. venustum	15,779	27	1/2 or less
S. tuberosum	149	21	1/2 or less

Seasonal Variation of Parasitism by *Sperchon* Larvae

Algonquin Park

In 1947 *Sperchon* larvae were first seen on emerging adult chironomids on June 20, and first on adult simuliids on June 27 in daily collections from an emergence cage over a square yard of Costello Creek. As can be seen in Fig. 1, the mite infestation began long after the beginning of emergence of black flies, and its peak was later (July 6). The emergence peak was influenced most by *S. venustum*, which made up 68% of the total collections. If we examine the emergence more closely we find that the mite infestation

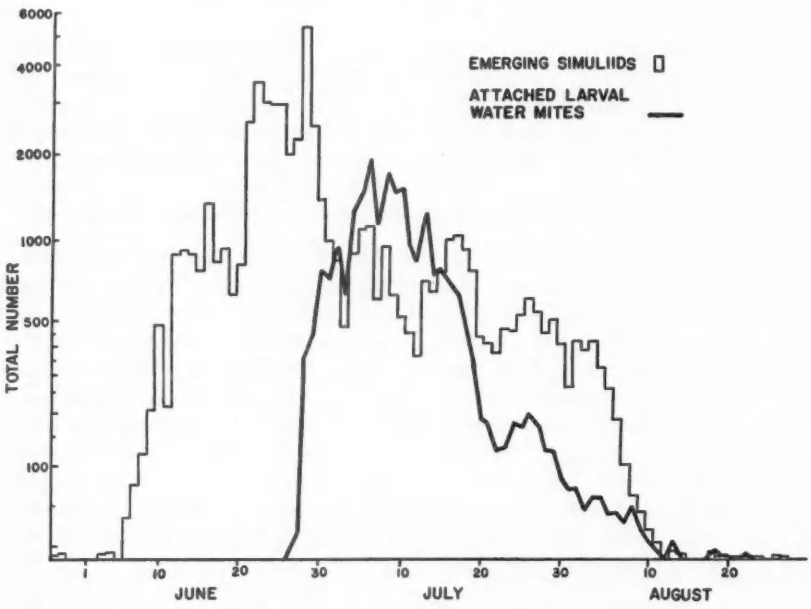

FIG. 1. The seasonal change in the larval mite infestation on adult black flies in relation to the seasonal change in the emergence of the adult black flies. Emergence was judged by daily collections from a cubic yard, 20-mesh per inch, screen cage placed over Costello Creek from May 28 to August 27, 1947.

follows the emergence of *S. decorum* females (Fig. 2), one of the more numerous and most heavily infested species. Mites on females of *S. decorum* made up 61% of the total mites collected, although females of *S. decorum* comprised only 9% of the emergent black flies (Table I). In nearly all cases when there was a peak in the emergence of *S. decorum* females, there was a corresponding peak in the total number of larval mites attacking but as one would expect, the number of mites per fly usually decreased to a minimum at these times (Fig. 2). The mite infestation on all black fly species ceased on August 22, although black flies continued to emerge until August 27. No mites were found on emerging midges at the same station after August 26.

In 1946 the mite infestation on emerging black flies occurred from June 20 to August 3 at Costello Creek, although simuliid emergence continued from May 26 to August 25. In 1955 netting collections over the North Madawaska River included a number of recently emerged black flies, and mites were found on netted simuliids from May 23 to July 18, and also one on an emerging female of *S. decorum* on October 9.

Baker Lake, 1949

Only the *Cnephia* sp. occurred in enough numbers to warrant presenting the seasonal infestation by larval mites. The infestation extended from

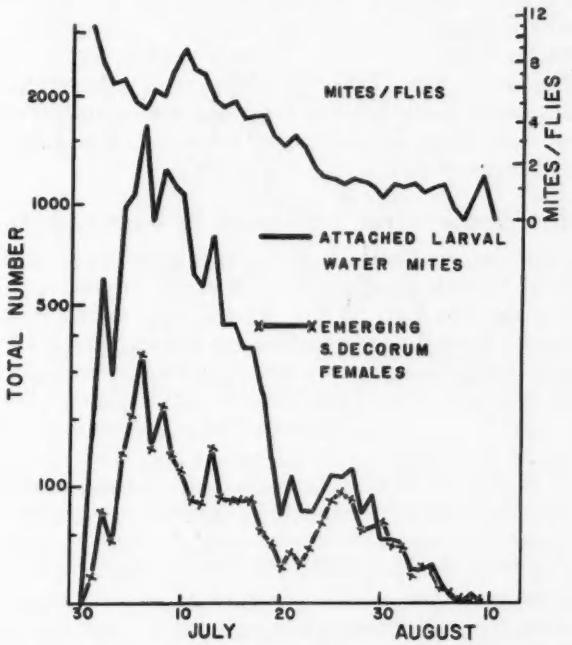

FIG. 2. The seasonal change in the total larval mite infestation on females of *S. decorum*, in the average number of mites per female, and in the total number of female flies emerging daily from a square yard of Costello Creek from May 28 to August 27, 1947. (Females of *S. decorum* emerged from June 6 to 24 but none were infested.)

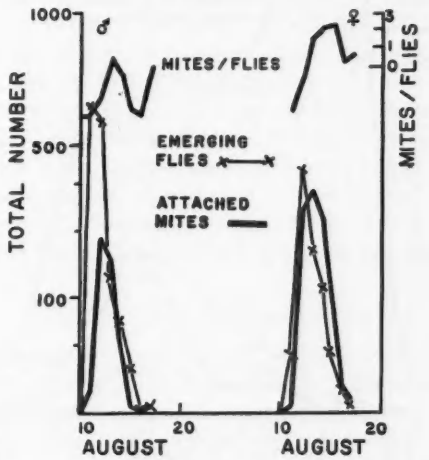

FIG. 3. The seasonal change in the total larval mite infestation on males and females of *Cnephia* sp., in the average number of mites per fly, and in the total number of flies of each sex emerging daily from a square yard of stream bottom at station No. 1, Baker Lake, Northwest Territories, from July 24 to August 19, 1949.

August 11 to 19, being a month later than the peak of infestation in Algonquin Park and also later than the infestation at Churchill, which extended from July 10 to August 11, 1948 (Table II), with a peak in late July. The peak in mite attack on each sex followed by a day the maximum emergence of flies (Fig. 3). The number of mites per fly reached its peak after the simuliid emergence had begun to decline.

Diurnal Changes in the Infestation by Larval Water Mites

Hourly collections were made from the emergence cages on June 28 and July 8, 1947, at Costello Creek. On the first date a good emergence of *S. aureum* was in progress and the diel change in parasitism by larval water mites could be compared with the emergence of this species of black fly (Fig. 4). Although most of the emerging flies were *S. venustum* and only 2% were *S. aureum*, 65% of the mites were found on the latter species. The change in infestation, both total mites and average number of mites per fly, followed the emergence of *S. aureum*, reaching a peak in midafternoon.

On July 8, *S. decorum* females were emerging in good numbers and were being heavily parasitized by water mites (Fig. 5). Seventy-two per cent of the mites occurred on females of this species, although the latter made up only 25% of the total emergence for the day. The change in mite infestation again followed closely the hourly emergence of this single species, i.e., *S. decorum* females, the peak in this case being in the late morning with a smaller rise in the late afternoon.

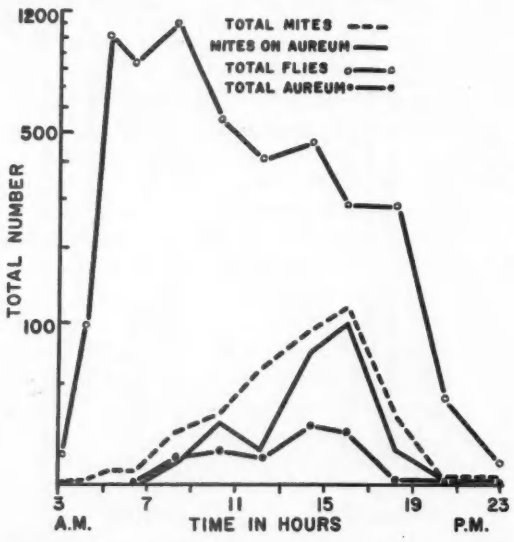

FIG. 4. The diurnal change in the total larval mite infestation on adults of all simuliid species and on those of *S. aureum*, and in the emergence of adults of all species and those of *S. aureum* on June 28, 1947, from a square yard of Costello Creek.

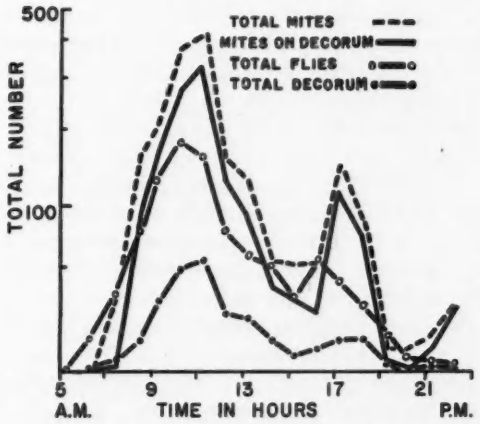

FIG. 5. The diurnal change in the total larval mite infestation on adults of all simuliid species and on females of *S. decorum*, and in the emergence of adults of all species and of females of *S. decorum* on July 8, 1947, from a square yard of Costello Creek.

Incidence of Mites on Simuliids Netted Away from the Stream

Netting collections of female black flies were checked for mites from June 27 to August 28, 1947. Near Costello Creek these included females of the following species: *Prosimulium decemarticulatum* Twinn 4, *Cnephia mutata* Malloch 4, *Cnephia subexcisa* 1, *S. latipes* 2, *S. decorum* 66, *S. tuberosum* 1, *S. venustum* 307, and *S. vittatum* 5. Also a few specimens of *decorum*, *latipes*, and *venustum* taken while landing and biting were examined. No larval mites were found on any of these flies. Mosquitoes in the same collection were infested with *Thyas* larvae (determined by Mitchell, 1957) from June 30 to July 10, one with 18 mites being netted on July 8.

On July 5, 1950, a female of *S. parnassum* Malloch bearing a mite was netted near Lake Sasajewun dam, Algonquin Park. Females of *S. venustum* with one to three mites were netted on June 2, 9, and 18, 1951, near the same place. Near Lake Sasajewun and Lake of Two Rivers, Algonquin Park, about 1% of females of *S. venustum* netted from May 28 to July 1, 1955, harbored from one to four mites (two to three mites per 100 flies).

Mites on Ovipositing Flies

Mites were not found on a number of ovipositing females of *S. venustum* that were collected from June 3 to August 11, 1947, at Costello Creek. Females of *S. decorum* and a few of *S. vittatum*, with mature eggs, were netted from June 2 to October 9, 1955. These were parasitized by *Sperchon* larvae and presumably were ovipositing or about to begin. About 5% of the 1500 *S. decorum* females netted were parasitized and the ratio of mites to flies was only 7:100. Oviposition by the black fly may aid in dislodging mites, which

may have partly released themselves already. *S. decorum* more often oviposits by tapping the tip of the abdomen on the water surface, the pressure releasing one or more eggs (5). This action may be important also in dislodging the larval mites.

Larval mites were also found stuck upside down on egg masses of black flies that oviposit on water-lapped surfaces, i.e. four on the evening of July 12, 1947, in Costello Creek on eggs of *S. venustum*; three at noon on June 9, 1952, in the Madawaska River (Whitney, Ontario) on the eggs of *S. vittatum*; one on the morning of June 27, 1953, in Ox-tongue River just below Tea Lake Dam on eggs of *S. vittatum*. These mites were apparently pulled from the fly's venter by the sticky egg matrix as the fly crawled over freshly laid eggs. It was noticed that the struggling larval mites, stuck upside down in the sticky egg matrix, were gradually loosened and in a few minutes washed free as the egg mass was lapped by the water. Hocking and Pickering (9) reported that at Churchill, Manitoba, many ovipositing females of *S. venustum* on July 16, 1951, had larval mites attached to them, one with as many as six in the cervical region. The return of water mites to the water by ovipositing flies was shown also for the chironomid *Cricotopus biformis* Edwards (12) and for the mosquito *Taeniorhynchus africanus* Theobald (6).

Observations on the Life History of the *Sperchon* Mites Attacking Black Flies

Eggs of *Sperchon* sp. were found first in 1955 on July 5, on a rotten stick in the North Madawaska River just below the sluiceway at Lake Sasajewun dam, but they may have been present earlier. They were observed again on July 13 and 17, and more may have been laid later, because many patches of mite eggs were found on August 6, 1955, in the Muskoka River at Bala, Ontario, but by September 4, no eggs were found at Lake Sasajewun dam. Eggs were laid in groups; three groups observed on July 13 comprised 11, 17, and 18 eggs respectively. The eggs were red like the other stages.

Eggs collected on July 13 were kept under $\frac{1}{4}$ in. of water in a watch glass in the laboratory at a mean temperature of 66° F (46–89° F). They began to hatch on July 24, or a day or two earlier, and hatching ended on July 30, when about half the 47 eggs had hatched (Table VII). In Sweden, eggs of *Sperchon squamosus* Kramer, collected on April 29, 1921, from a warm stream, all hatched within 12 days and those of *S. glandulosus thienemanni* Koen. within 23 days (14), but the actual temperature during incubation was not given.

The larval mites appear to be free-living for a variable period of time, creeping on the bottom of the stream rather than swimming. In many simuliid species the larvae, living in running water, spin a well-defined slipper-shaped cocoon before pupating. The cocoons provide some protection for the parasitic larval mites. Grenier (7) indicated that the larval water mites appeared to attack the respiratory filaments and abdomen of pupae of three simuliid species in France, those with more open cocoons having a greater

TABLE VII

The number of the 47 eggs of *Sperchon* hatching on certain dates. These were collected on July 13, 1953, in North Madawaska River, just below Lake Sasajewun dam, and kept in the laboratory at a mean temperature of 66° F. No further hatching occurred from July 31 to August 7

Date	Number hatched
July 23	–
24	6
25	–
26	5
27	4
28	4
29	2
30	2
31	0

infestation. Although he found no lesions, he remarked that the pupae were often shrunken with large larval mites inside the cocoons. In Algonquin Park, larval mites were seen crawling over, and even deep within, cocoons of *S. aureum* and *S. decorum* in July but shrunken pupae were not conspicuous and whether the larval mites were feeding on the pupae was not established.

The adult black fly, just before emergence, is surrounded by a layer of gas. Once the pupal skin splits, the adult takes several minutes to pull itself clear, after which it is carried through the turbulent current to the surface. Emergence takes 8 minutes in *P. hirtipes* (s.l.) (3) and 3 minutes in *S. venustum*. Apparently this gives ample time for the larval water mites to transfer themselves from the cocoon to the adult black flies. The efficiency of this transfer is demonstrated by the finding of as many as 32 larval mites on one adult female of *S. decorum*.

The larval water mites that transfer to emerging adult simuliids attach themselves to the soft parts of the fly, usually in places where there is least danger of their being brushed off. Most are found on the ventral surface of the first two abdominal segments. Sometimes a few are found on the underside of the thorax, their heads down between the coxae of the legs, the first and third pair having more mites than the second pair of legs. They are found occasionally between the head and prothorax forming a collar with their heads inwards. Ten mites on two emerging flies collected on June 24, 1946, and July 8, 1947, averaged 0.26 (0.17–0.34) mm in width.

The infestation of larval *Sperchon* began in late June and continued on black flies for 45 days in 1946 and 57 days in 1947, and on chironomids for 68 days in 1947. In 1955 it looked as if the infestation on black flies began in late May and continued, although with diminishing intensity, into October. Whether the early infestation was the result of overwintering larvae or eggs, or of eggs newly laid in the spring has yet to be established. But later infestation appears to result from newly laid eggs that hatch after a 2-week incubation.

Although the emergent black flies were often heavily infested, the number of mites on flies netted away from the stream, usually some days after emergence, was much lower. However, some mites remained attached to the female flies until the flies oviposited, at which time the larval mites returned to a free-living existence in the water. When there are only one to three mites per fly they are usually larger than when many are present. A larval mite on an ovipositing female of *S. decorum* on June 28, 1946, was 0.51 mm in width and two on simuliid eggs averaged 0.59 mm in width. In 1947 in Algonquin Park the first larval mites on emerging females of *S. venustum* occurred on June 28 and the first seen on *venustum* egg masses on July 12, an interval of 14 days, which may be roughly the length of the parasitic period in the life cycle of *Sperchon*. The time taken for the free-living larval mites to pass through the nymphal stage to the adult is unknown.

Adults of *Sperchon* have been seen on June 25, 1946, and August 9, 1947, in Costello Creek; from May 24 to July 17, 1955, in the North Madawaska River, just below Lake Sasajewun dam; and on August 6 and September 4, 1955, in Muskoka River, just below the dam at Bala, Ontario. An adult collected in Costello Creek on June 15, 1955, measured 0.88 mm in width, about twice that of larval mites at the end of their parasitism.

Both larval and adult *Sperchon* appeared in streams of Algonquin Park in May. Whether the larvae hatched from overwintering eggs or overwintered themselves is unknown, but some were at the parasitic stage in mid-May. Possibly the adults laid eggs in May or early June, although our irregular observations disclosed eggs first in early July. The peak parasitism occurred during July, although scattered observations of larval mites on black flies were made from mid-May to early October. Possibly the July peak in parasitism resulted from eggs laid by overwintering adults, and the irregular parasitism at other times resulted from the overwintering larvae or eggs, which may have produced a diffuse, attenuated second generation.

Farther north the maximum infestation occurred later in the season, as judged by a comparison of the data from Algonquin Park, Churchill, and Baker Lake.

Discussion

The parasitism on black fly adults may be an important, and even essential, part of the life cycle of this species of *Sperchon* (possibly *S. jasperensis*). However, Grenier (7) in France noted black fly pupae being parasitized, which indicates that in the species of water mite there, the cycle might be completed without the larval mites leaving the water.

Ten species of black fly were seen to be attacked by *Sperchon* larvae and only two somewhat related species, emerging during the infestation period, were not, namely *C. emergens* and *C. mutata*. However, of the simuliid species attacked some were much more heavily infested than others and usually the females much more than the males. The species most heavily attacked on emergence were usually those with the most stored nutrient in

fat bodies or in almost mature eggs. There may be more than one factor related to the more frequent selection of certain simuliid species by the larval mites. It is possible that there may be some chemical factor, released as the pupal skin splits, which stimulates larval mites to congregate nearby or more readily to attack emerging adults, because during a single day sometimes a higher percentage of adult flies is parasitized when more flies are emerging (Fig. 4). Mites are larger when fewer are present per fly and may double in size during their parasitic stage. Nevertheless the advantage to the larval mites of selecting certain simuliid species may be more than being on a host with greater nutrient. The amount of activity of the host and the length of the interval between the host's emergence and oviposition may be of much more ultimate importance to survival of the larval mites. Certain black fly species range some distance and may be carried several miles by the wind. This is less frequent in the non-bloodsucking species, which, although they may be attracted by nectar in flowers, do not require the blood of a homoithermic vertebrate. In species such as *C. dacotensis*, *S. aureum*, and *S. decorum*, with stored nutrient in newly emerged females, less general activity, and a shorter interval between emergence and oviposition, more larval mites per female fly would be returned to the water. Also simuliid *females* because of their oviposition habits provide a much better probability for the return of larval mites to the water than do males.

The larval mites clinging to the cocoon easily transfer themselves to the simuliid adult as it emerges from the pupal skin, most usually attaching themselves between the sclerites of the venter. Although newly emerged flies sometimes carried 10–32 *Sperchon* larvae, those netted away from the stream, presumably a day or more after emergence, had fewer than 10 larval mites, usually 1–3. This may indicate that mites drop off or are dislodged because of the activity of the fly, of greater effect in simuliid species requiring a blood meal. On the other hand there is another, though less probable, explanation for the low incidence in these cases. It may be the result of heavily infested flies being so debilitated that they fly and feed less, and more readily die. Gillett (6), working with mosquitoes, agrees that this latter explanation is less probable because it makes the life cycle of the mite too precarious, and Abdel-Malek (1) reported that up to 15 larval water mites per mosquito apparently did not affect the activity or longevity of the fly but that the fecundity of the female mosquitoes was reduced. On the other hand, Uchida and Miyazaki (1935) in Münchberg (17) found that with increased numbers of mites the longevity of mosquitoes was shortened. Whether some larval mites after being dislodged or after the death of the black fly could survive long enough to crawl to the water is yet to be shown. Lundblad (14) said that he had not seen larvae of *S. squamosus* away from the water. In any case the much lower frequency of mites on flies netted some time after emergence and at the time of oviposition indicates that only a small percentage of the mites, transferring to emerging adult simuliids, reach the water again. There is no evidence of black flies returning frequently to

the breeding site to drink water, so that few mites would be returned to the water in this way. The only observations on the return of larval water mites to the water are those when female black flies are ovipositing.

Leger and Motas (12) found that well-fed larvae of *Piona* mites soon become nymphs after leaving the chironomid host but if insufficiently fed, may die and be soon covered with *Saprolegnia*. Nymphs prey on the newly hatched chironomid larvae and 20 days after leaving the host they become mature. Further studies are needed to determine the length of the free-living stages of the *Sperchon* mite of black flies, how it overwinters, and how many generations per year it has.

The most important blood-sucking simuliid species are among the least attacked by *Sperchon*. Usually *P. hirtipes* (s.l.) completes emergence before the larval mites are abundant. *S. venustum* and *S. parnassum* with small fat bodies in newly emerged females are much less attacked than some other species. Only the population of *S. decorum*, usually of secondary importance to humans in Ontario, appears as if it might be controlled at times by the parasitism of larval water mites.

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OBSERVATIONS ON AMOEBOID MOTION OF LIVING HAEMOCYTES IN THE WING VEINS OF BLABERUS GIGANTEUS (L.) (ORTHOPTERA : BLATTIDAE)[1]

J. W. ARNOLD[2]

Abstract

Haemocytes with finely granular cytoplasm moved independently in partly occluded wing veins of *B. giganteus* by two related but fundamentally distinct methods: (*a*) typical amoeboid motion that occurred generally on unobstructed vein walls and was characterized by cytoplasmic streaming into amorphous pseudopodia, and (*b*) atypical amoeboid motion, without visible cytoplasmic streaming, that involved the projection of hyaline ectoplasm into tactile and adhesive filiform or lamellar pseudopodia. By the atypical method the cells became oriented and entered into confined spaces. Speed of movement varied but approximated 5 microns per minute with typical amoeboid motion and 3.5 with the atypical during pronounced cell migration. Haemocytes with hyaline cytoplasm, coarsely granular cytoplasm, or cytoplasm that contained numerous globules moved comparatively little and only by the atypical method.

Introduction

Although the ability of insect haemocytes to move independently may be deduced from their phagocytic and wound-healing activities, movements other than minor ones such as changes in cell form have not been observed directly. In 1957 prolonged observation by the writer on living haemocytes in the wing veins of the cockroach *Blaberus giganteus* (L.)(1) provided no evidence of amoeboid motion and seemed to confirm the view, sometimes expressed, that independent movement by these cells is negligible except perhaps under certain conditions of stress.

During additional study of blood circulation in the wings of *B. giganteus*, haemocytes were frequently observed moving independently in partly occluded veins of apparently healthy adults. These observations contradict the aforementioned view. They also help considerably in understanding the significance of haemocyte form and structure as related to function and to classification. This paper describes the nature of the independent movements.

Materials and Methods

Adults of *B. giganteus* were found to be especially suitable for studies of this kind and were used exclusively. The cuticle of the wings remains comparatively clear throughout the insect's life, the blood circulation through the wing veins is relatively steady, and the haemocytes are large. Furthermore, the roach is lethargic and usually remains quiet for long periods during microscopic examination of the wing.

The roaches were obtained periodically from the Pesticide Testing Laboratory, Canada Department of Agriculture, Ottawa, and maintained at room

[1]Manuscript received February 21, 1959.
Contribution No. 3908, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Entomology Laboratory, Ottawa, Ontario.

temperature and approximately 70 per cent relative humidity on a diet of dog biscuits and bananas.

Methods for observing and photographing the living haemocytes were similar to those described previously (1) with only the following significant changes: A magnification of 1350× (oculars 15×; objective 90×) was used almost exclusively for observation and photomicrography. This was found to be advantageous in view of the slowness of amoeboid motion and the almost invisible, hyaline nature of the pseudopodia in some cases. Illumination for photomicrography was provided by a Leitz Multiblitz-Micro Electronic Flash Unit (E. Leitz Ltd., Germany).

Observations and Discussion

Independently moving haemocytes were observed in a number of adult roaches that varied in age from about 3 weeks after the final nymphal molt to 6 months. Presumably they are active throughout the insect's life. They were seen most frequently in the old specimens and especially in partly occluded wing veins where the haemolymph flow was retarded and cell circulation restricted or absent. It was not determined whether these conditions induced cell movement or simply favored its observation.

Cells with finely granular cytoplasm were by far the most active of the haemocytes and received special study. Other types, with cytoplasm that was hyaline, coarsely granular, or that contained numerous globules showed slight or only moderate ability to move under these conditions and received less attention. Consequently the description of cell movement pertains to finely granular cells unless otherwise stated. This separation of the haemocytes on the basis of cytoplasmic structure is not intended to be a classification system. It is simply a convenient and in this case a pertinent way of distinguishing among a complex of cells that may be generally termed plasmatocytes.

The haemocytes moved independently by two related but distinctive methods, typical amoeboid motion and atypical amoeboid motion, that usually functioned in different circumstances.

Typical Amoeboid Motion

Movement by this method resulted from the streaming of the cytoplasmic mass into amorphous pseudopodia (Fig. 1). The action sometimes began spontaneously and, in the case of one cell under observation, was initiated by the mechanical pressure of another cell squeezing past. Typically it resulted in cell migration at a steady and comparatively rapid rate. The average speed at room temperature (approx. 25° C), from measurements of the distances travelled in known time by five different cells, was approximately 5 microns per minute. The greatest distance that a cell was seen to travel without interruption was 150 microns. Like an amoeba the haemocytes altered course at obstacles and occasionally reversed direction. They were usually seen moving in this way in occluded veins where the lumen

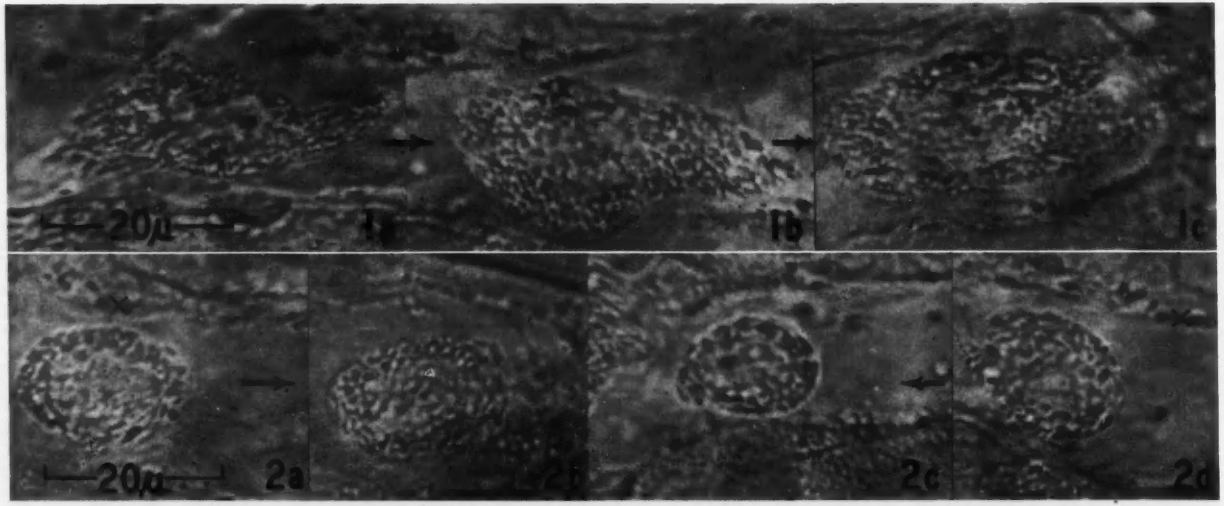
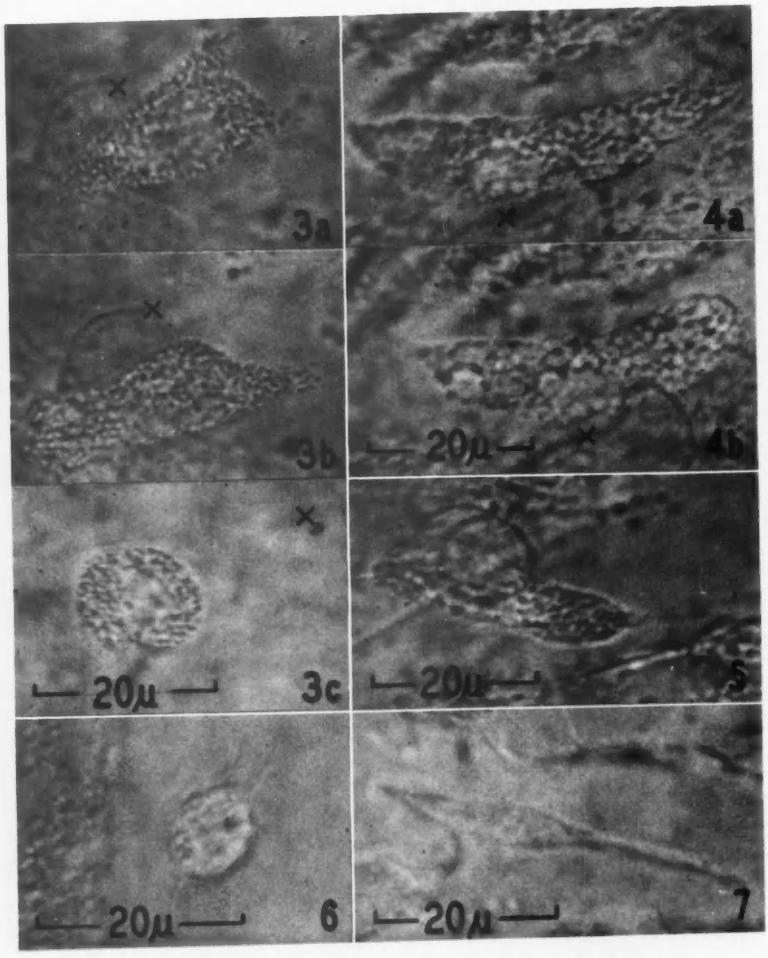


FIG. 1. Haemocyte migrating by typical amoeboid motion into a dead-end vein. *a*, *b*, and *c* show the cell at different positions in the vein. Subsequently the cell reversed its direction of flow and moved out of the vein. Total elapsed time *a* to *c*, 15 minutes.

FIG. 2. Haemocyte migrating by atypical amoeboid motion into the same vein. Hyaline, lamellar pseudopodium not visible in the photomicrographs. *a* and *b* show the cell entering the vein, *c* and *d* leaving it. × is a point of reference on the vein wall. Note general maintenance of cell form and elongation of granules at advancing edge. Total elapsed time *a* to *d*, 30 minutes.

PLATE II



beyond the entrances was mainly unobstructed. Presumably this is the typical method for cell migration on open surfaces. Migration was followed by a period of quiescence or an interval when the cell was mainly stationary and extending in various directions by atypical amoeboid motion.

Atypical Amoeboid Motion

The nature of this process was obscure, but it involved the separation of the cell's cytoplasm into an outer hyaline ectoplasm or plasmagel which was sometimes limited to the front of the moving cell, and an inner granular and apparently more fluid endoplasm or plasmasol. Movement was accomplished by projection of the hyaline layer alone into filiform or lamellar pseudopodia, depending upon the cell's position, and not usually into the amorphous pseudopodia that distinguished typical amoeboid motion. Haemocytes occasionally migrated on unobstructed vein walls by this method but were not readily perceived due to their slow rate of speed and the short distances travelled or to the rapid change to typical amoeboid motion. More frequently they changed position and traversed short distances across gaps, into narrow portions of veins or into confined spaces among groups of cells where considerable constriction was required to gain entrance. Analogous motion of invertebrate amoebocytes associated with somewhat similar pseudopodia was suggested by Fauré-Fremiet (2) and others.

When the cells were migrating appreciably on unobstructed surfaces by this method (Fig. 2) the hyaline pseudopodia were lamellar, often with scalloped edges, and were especially difficult to see and to photograph. The granular endoplasm did not stream or enter the pseudopodia but the adjacent granules were noticeably elongate and some turbulence or tension seemed to occur there. There was usually little change in the form of the cells except when they squeezed past obstructions. Migration was comparatively slow. The average speed, from observations on four cells that travelled appreciable distances, was 3.5 μ per minute.

When the cells were changing position slightly or moving across gaps or into confined spaces the pseudopodia were typically filiform (Fig. 5). They usually developed from the tips of fusiform or fusiformoid cells but emerged elsewhere from these and from variously shaped cells. Consequently, although

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FIG. 3. Haemocyte changing form. *a*, extending filiform pseudopodia in three directions simultaneously. *b*, same cell 5 minutes later retracting upper pseudopodium and with endoplasm streaming mainly into broadening pseudopodium at left. *c*, same cell 5 minutes later with all pseudopodia retracted has released attachment to vein wall and drifted slightly from original position. × marks a point of reference on the vein wall.

FIG. 4. Haemocyte with numerous globules in cytoplasm. *a*, cell with hyaline, filiform pseudopodia to vein wall. *b*, same cell, 6 hours and 50 minutes later, has retracted right pseudopodium and moved slightly from point of reference ×.

FIG. 5. Haemocyte that was previously moving to right by filiform pseudopodium now moving to left by lamellar pseudopodium in atypical amoeboid motion. Remnant of filiform pseudopodium remains at right.

FIG. 6. Haemocyte with several hyaline pseudopodia from its surface.

FIG. 7. Fusiform hyaline haemocyte with filiform pseudopodium to right. Branched tip of pseudopodium shows slightly.

one or two pseudopodia were typical, cells with three or more were not rare (Fig. 3). The pseudopodia usually extended about one-half the cell's length and occasionally became much longer. They were hyaline but usually contained one granule or several in a small group constantly jostling at about the mid-point. They were turgid, tactile, and especially when extended in a clear space, oscillated slowly within an arc of approximately 15 degrees. They adhered readily to objects they touched but released easily. Occasionally when their attachment to an object became separated they retracted rapidly into the cell body (Fig. 3) as if they had been under considerable tension. At other times they retained their form even when the cell moved in the opposite direction (Fig. 5). For the most part they were only indirectly involved in migration. They were the means by which the cell's movement was directed, particularly into narrow spaces where the typical, less turgid and apparently less sensitive pseudopodium would scarcely penetrate. Although they seemed capable of slight contraction to draw the cell toward their point of attachment, the principal means of progression was by further extension of the pseudopodium as endoplasm moved into it. Initially at least the endoplasm did not stream into the pseudopodium. It merely followed the advancing hyaline ectoplasm and conformed to its outlines. These outlines usually became modified as the cell advanced. The base broadened with the inflow of endoplasm, or the pseudopodium became lamellar, still with an advancing front of hyaline ectoplasm, or changed completely to the typical amorphous, streaming pseudopodium. Under these conditions the haemocytes changed form and position slowly and continually, extending first in one direction and then in another. Presumably this is the manner in which haemocytes become insinuated among other tissues as reported by Wigglesworth (8).

Similar filiform pseudopodia developed in hyaline cells (Fig. 7), granular cells, and cells with numerous globules (Fig. 4) but they seldom caused appreciable movement. Usually they extended and attached to an object, remained thus without apparent change for periods up to several hours, and then retracted into the cell body. The pseudopodia of the hyaline cells were somewhat different from those of the other types and warrant further study. Their tips were often expanded and sometimes appeared to be branched. They were occasionally responsible for slow cell movements over comparatively small distances.

Very adhesive, hyaline, filiform, sometimes branched pseudopodia that resembled but seemed to be fundamentally different from those described previously occurred over the surface of certain distinctive cells (Fig. 6). These cells were small, spherical, hyaline, and often vacuolate. They occurred in small numbers in the circulating haemolymph, especially in old adults. The pseudopodia frequently caused cell clumping due to their adhesive nature and the cells may therefore be analogous to those that Grégoire found responsible for coagulation in numerous species (3). Similar cells have been noted by several authors including Jones (5) and Rizki (6), especially in wet

smear and in vitro preparations. Superficially they appear to be degenerating forms with cytoplasmic eruptions.

The changes in cell form associated with independent movement probably account for some of the discrepancies in haemocyte classifications. Cells flowing by typical amoeboid motion bore little superficial resemblance to those that were extending filiform pseudopodia, even though both activities alternated in a single cell. Likewise a cell without pseudopodia differed markedly from one with several short extensions and/or one with one or two extremely long ones. Various authors have interpreted these features differently in other insects. Some, including Wigglesworth (8) and Smith (7) have recognized them as modifications of a single multifunctional type of cell. Others, for example Yeager (9) and Jones (4), have categorized them, either for convenience or to signify possible functional differences. The present observations suggest that cell form should not be a criterion for haemocyte classification in this insect and probably not in others. Further observations are required to determine the significance of other characteristics, such as the nature of the nucleus and cytoplasm, that seem to be more stable or at least more apt to show predictable and progressive biochemical and functional changes associated with ageing.

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NOTES

PARASITISM OF UNWEANED SHREWS BY THE CESTODE HYMENOLEPIS FALCULATA

W. J. Hamilton, Jr., and G. A. Schad

On August 27, 1947, W. J. Hamilton, Jr., and W. J. Hamilton, III, collected a nursing *Sorex cinereus cinereus* with her five young in a nest of dried *Vallisneria* beneath driftwood at Southampton, New York.

Examination of three young, estimated at 15 to 16 days, revealed the presence of the cestode *Hymenolepis falculata* Rausch and Kuns in the stomach and fore-intestine. The presence of clotted milk in the stomach and the absence of other food is evidence that the shrews were not weaned and thus a question arises as to the mode of entry of a parasite which probably uses an arthropod vector. While it is realized that an arthropod intermediate may not be necessary and that prenatal infection could possibly occur, a simpler explanation is suggested by shrew behavior. These shrews commonly practice refection, eating their own rectal contents. It is possible that young shrews feed from the anus of the nursing parent. Certainly they would have opportunity to do such if the mother practices this in the nest. Since the infective stages of intestinal parasites are frequently swept through a host before they can succeed in establishing themselves, it is suggested they may obtain a second chance at infection if ingested by the nestling shrew feeding on adult faeces.

The cestodes from the individual shrews (five nestlings of which three were examined) were combined in one lot and, therefore, the number of worms per host is unknown. However, 13 scoleces were present and the total number of worms based on the number of strobilae and parts of strobilae is estimated to be 17 to 18. Thus, the average would be approximately six tapeworms per shrew.

The cestodes in this lot differ from *Hymenolepis falculata*, as previously described, in that they have fewer hooks. Ten hooks were counted instead of the usual twelve.

The shrews are alcoholic specimens Nos. 5113–5117 Cornell University Mammal Collection. The cestodes are in the collection of the Institute of Parasitology (Collection No. 125/2/24).

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Department of Conservation,
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THE STOMACH CONTENTS OF HARP SEALS (PHOCA GRÖENLANDICA ERXLEBEN) FROM THE MAGDALEN ISLANDS, QUEBEC

BETTY J. MYERS

From March to May 1956 I examined the stomach contents of 195 adult harp seals (75 females, 120 males) whelping in the Magdalen Islands area. The stomachs of 107 (55%) contained food, the remaining (mostly of seals taken in March and April) 88 (45%) being empty. The stomach contents consisted of: herring (*Clupea harengus* Linnaeus, 1758) in 58%; flatfish sp. in 15%; redfish (*Sebastes marinus* (Linnaeus, 1758)), in 4%; witch (*Glyptocephalus cynoglossus* (Linnaeus, 1758)), in 1%; plaice (*Hippoglossoides platessoides* (Fabricius, 1780)), in 1%; and sea mouse (*Aphrodita* Linnaeus) in one stomach which contained no other food. One stomach contained the remains of crustacea which had probably been released from the digested stomach contents of larger organisms.

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INSTITUTE OF PARASITOLOGY,
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GREGARINES FROM LABORATORY COLONIES OF FLOUR BEETLES, TRIBOLIUM CASTANEUM HERBST AND T. CONFUSUM DUVAL, AT MONTREAL

MARSHALL LAIRD*

During February, 1959, it became evident that protozoan parasites were causing mortality in laboratory colonies of *Tribolium castaneum* Herbst and *T. confusum* Duval in the Department of Zoology, McGill University. First impressions were that pathogenicity was higher among black mutants of the two hosts than in the normal red strains. Professor John Stanley is to be thanked for forwarding moribund larvae for identification of the organisms discussed herein.

Fresh material in normal saline was examined by phase contrast. Permanent preparations were made by fixing cover slip smears in Davis' modification of

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Worcester's fluid (1) and staining with Heidenhain's iron haematoxylin, and by sectioning at 10 μ and staining with Ehrlich's haematoxylin and eosin.

Huge numbers of the trophozoites, schizonts, and spores of a schizogregarine flooded from crushed larvae. Dissection and sectioning revealed that the fat body is the primary site of infection, that involvement of the haemocoele is common, and that muscle may be invaded as well. The vermiform trophozoites and the occurrence of macronuclear and micronuclear schizonts refer the parasite to the family Ophryocystidae as defined by Weiser (8, 9). A well-known and highly pathogenic member of this family, *Mattesia dispora* Naville, has fusiform spores with thickened poles very like those of the present species. As the former organism does not exhibit close host specificity, parasitizing grain-dwelling moths of at least two genera and cucujid beetles as well (3), it was at first suspected that it was responsible for the McGill infections.

However, only 1 or (usually) 2 spores develop within each gametocyst of *M. dispora*, while from 4 to 16 (usually 8 or 10) characterize the *Tribolium* parasite. This excludes from further consideration both *M. dispora* and *Farinocystis tribolii* Weiser, which infects *T. castaneum* and *T. confusum* in Czechoslovakia but in which the usual spore number is 32 (7, 8). A further member of the Ophryocystidae, *Triboliocystis garnhami* Dissanaike (2), was recently described from *T. castaneum* in England, and there is close similarity between the various life-history stages of this species and the present one.

Gametocysts in the type material of *T. garnhami* contained from 3 to 10 (usually 8) spores, which in the fresh state measured from 13.3 to 14.3 μ by from 6.6 to 7.7 μ (2). Fifty fresh spores from the McGill larvae measured from 12.9 to 14.3 μ by from 6.7 to 7.8 μ (av., 13.5 by 7.2 μ), the same number of slightly shrunken, fixed, and stained ones measuring from 11.4 to 13.5 μ by from 5.7 to 6.5 μ (av., 12.2 by 6.2 μ). Agreement extends to the posteriorly broadened and somewhat crescentic shape of the trophozoites and to the details of schizogony and gametogony. The Montreal parasite is thus identified as *Triboliocystis garnhami*, the strain differing from the type material in the rather wider range in the number of spores formed per gametocyst.

Gregarines also proved to be present in the intestine of the larvae examined. The species concerned, a eugregarine, has septate trophozoites and thus belongs to the tribe Cephalina Delage; and as the sporadins are bi-associative and both primite and satellite are septate, it is referable to the family Gregarinidae Labbé. The protomerite of the primite is dome-shaped, and ranges from as wide to about twice as wide as long. Posteriorly the deutomerite of the primite is truncate, and the protomerite of the satellite is approximately rectangular. There is slight if any constriction at the septum, and the deutomerite of the satellite is broadly rounded posteriorly. The nucleus is spherical and has a single large endosome. This parasite displays considerable polymorphism, as is seen from the measurements of five living

associations selected at random and here arranged in ascending order of size (measurements in microns):

Primite					
Length of protomerite	8.2	14.4	10.9	10.9	14.9
Breadth of protomerite	12.3	14.4	18.6	18.5	19.2
Length of deutomerite	91.5	95.6	114.0	134.3	179.1
Breadth of deutomerite	26.1	27.4	41.1	37.0	41.1
Total length	99.7	110.0	124.9	145.2	194.0
Satellite					
Length of protomerite	9.6	8.2	10.0	10.0	8.8
Breadth of protomerite	16.5	13.7	16.5	18.5	19.0
Length of deutomerite	102.8	109.0	105.5	142.5	107.2
Breadth of deutomerite	31.5	30.2	32.8	40.0	40.5
Total length	112.4	117.2	115.5	152.5	116.0
Total length of association	212.1	227.2	240.4	297.7	310.0

The ratio of the length of the protomerite: total length::1:7–1:15, and that of the width of the protomerite: that of the deutomerite::1:1.9–1:2.2. In all but size the organism bears comparison with *Gregarina minuta* Ishii (4), described from (*T. ferrugineum*) = *T. castaneum* in Japan. Watson (6) indicated that Ishii had confused two very different gregarines under this name, one of them lacking a protomerite in the satellite and being referable to the genus *Didymophyes* Stein. A protomerite was absent from the satellite of a few associated sporonts of the species under consideration, although these otherwise resembled the dominant form in all respects. A number of isolated sporonts were also seen. Watson retained the name *G. minuta* for a small species an association of which Ishii (4) had found to be only 118 μ in total length (although, judging from his figure *b*, there is close morphological agreement with the Montreal eugregarine). In size, the latter stands closer to *Gregarina polymorpha* Stein, the type host of which is another tenebrionid grain beetle, *Tenebrio molitor* L. Associated sporadins of *G. polymorpha* reach an over-all length of 350 μ, but are broad in proportion, their maximum breadth being 100 μ. Pending more detailed studies of G. *polymorpha* and *G. minuta*, which might conceivably prove that the latter name is synonymous with the former, and that Ishii's forms lacking a protomerite in the satellite were merely aberrant and not referable to *Didymophyes* at all, it is not proposed to do more than identify the present intestinal parasite of *Tribolium* as *Gregarina* sp.

T. confusum is thought to be a new host for both *Triboliocystis* and *Gregarina*.* Eugregarines are generally non-pathogenic, and the second parasite might well have been present in the McGill colonies for a considerable time. The schizogregarine, though, is presumed to be a recent introduction, probably through the medium of spore-contaminated flour. Dissanaike's observations on the pathogenicity of *T. garnhami* suggest that the consequences are likely to be disastrous insofar as the regular colonies are concerned. It should be noted by way of contrast that *Gregarina steini* Berndt—perhaps a synonym

*Since this was written, it has been found that there is in fact a record of *Gregarina polymorpha* from *T. confusum*.—Leonova, N. Influence of external factors on the intestine fauna of *Tribolium confusum* Duv. Trudy Sredneaziat. Gosudarst. Univ. s. 8–*a* **34** (1937).

of *G. polymorpha*, according to Watson (6)—is actually held to favor the rapid and healthy development of *Tenebrio molitor* (5). Whether or not this is so, it is obvious that the unpremeditated presence of any parasite, dangerous or otherwise, in laboratory colonies of insects, is prejudicial to the results of studies on population dynamics.

The possibility that the black mutants of *Tribolium castaneum* and *T. confusum* are more susceptible to *Triboliocystis* than normal strains stands in need of investigation. At the same time, the economic importance of the hosts is an incentive to intensive studies of an organism that might prove to have biological control significance. For these reasons the infection is to be maintained in a colony set aside for that purpose in the Department of Zoology, McGill University, from where cultures may be obtained on application by those interested.

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CORRECTIONS

Volume 36, 1958

Page 631. The caption for Fig. 1 should read "Time – percentage mortality curves for exposure to 45° C for liver-reared *P. affinis* larvae maintained at 23° C (●) and conditioned at 39° C for 2 hours (○)".

Volume 37, 1959

Page 18. In line 21 of the section *Abdominal Process and Caudal Spine*, "anteriorly projecting spines" should read "posteriorly projecting spines".

NOTES TO CONTRIBUTORS

Canadian Journal of Zoology

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